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Soft-Shell Clam Depuration
Digby, Nova Scotia

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Technical Reports

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ABSTRACT

Rowell, T.W., G. Robert, K.B. Swansburg and R. Davis. 1976. Soft-shell Clam Depuration, Digby, Nova Scotia. Fish. Mar. Serv. Res. Dev. Tech. Rep. 687: 121 p.

The controlled purification of bacteriologically contaminated shellfish through depuration has been widely recognized as a means of increasing shellfish production from the ever-increasing number of polluted estuaries. In the Maritimes, substantial soft-shell clam stocks in the Bay of Fundy region are presently unavailable for harvest due to extensive shellfish "closures". This report describes pilot depuration operations at Digby, Nova Scotia and the operational criteria and procedures necessary for reliable commercial depuration. A flow-through system using a Kelly-Purdy type U.V. sterilizer was utilized. This provided water of acceptable bacteriological quality.

Soft-shell clams with an initial faecal coliform MPN count of less than 2000 cleansed themselves to acceptable levels with 42 hours of depuration. A water flow-rate of 0.5 gpm per bushel (60 lbs) of clams was found optimal. Drainage of the depuration tanks and thorough hosing of the tanks, clams and depuration baskets were found essential. Citrate-utilizing bacteria of the Klebsiella, Citrobacter and Enterobacter types were demonstrated to be major contributors to the elevated FC counts obtained in the initial shellstock counts and in the treated shellfish during a brief period of the summer. These bacteria, while of doubtful significance to the public health, could, in commercial operations, lead to rejection of processed shellfish due to the unspecific nature of the standard 5-tube MPN test.

Based on an annual production of 9540 bushels, and dependent on factors included, it would cost between $1.04 and $2.15 per bushel for depuration.

Recommendations are made on general operational procedures, bacteriological techniques and sampling necessary to commercial operations, equipment design and means of increasing handling efficiency.

RESUME

Rowell, T.W., G. Robert, K.B. Swansburg and R. Davis. 1976. Soft-shell Clam Depuration, Digby, Nova Scotia. Fish. Mar. Serv. Res. Dev. Tech. Rep. 687: 121 p.

La purification contrôlée de coquillages contaminés par les bactéries via la dépuration est largement reconnue comme moyen

d'accroître la production en coquillages du nombre toujours grandissant d'estuaires pollués. Aux Maritimes, plus particulièrement dans la région de la Baie de Fundy, on ne peut récolter des quantités appréciables de myes dû à d'importantes "fermetures" de bancs de coquillages. Ce rapport décrit les opérations d'un plant-pilote de dépuration à Digby, Nouvelle-Ecosse ainsi que les critères opérationels et procédures qui seraient nécessaires à l'échelle commerciale. On a utilisé un système à circulation unique avec un stérilisateur à rayons Ultra-Violet de type Kelly-Purdy. Ce genre de traitement fournit une eau de qualité bactériologique approuvée.

Des myes dont le taux initial de colibacilles fécaux est de 2000 NPP ou moins réduisent leur degré de contamination à des niveaux jugés acceptables en-deçà de 42 heures de dépuration. On a trouvé que le meilleur courant d'eau est de 0.5 gpm par boisseau (60 lbs) de myes. On a jugé essentiel de drainer les bassins de dépuration et de nettoyer par arrosage les bassins et les paniers de depuration et les myes. Des bactéries utilisatrices de citrate telles Klebsiella, Citrobacter et Enterobacter sont en grande partie responsables des comptes élevés de colibacilles fécaux obtenus comme taux initial et taux traité pendant une courte période de l'été. De peu d'intérêt pour la santé publique ces bactéries pourraient occasionner le rejet de lots de coquillages dépurés à cause de la nature peu spécifique de l'analyse NPP 5-tubes.

Si on estime le rendement annuel à 9540 boisseaux, et dépendant de l'addition de certains facteurs, la dépuration d'un boisseau coûterait entre $1.04 et $2.15.

On inclut des recommendations sur les procédures opérationelles, les techniques bactériologiques et l'échantillonnage requis d'une opération commerciale, ainsi que sur la description de l'équipement et des moyens d'augmenter l'efficacité par mécanisation.

INTRODUCTION

This report describes and assesses the 1975 trials of a pilot plant for the depuration of soft-shell clams (*Mya arenaria*). The project was a co-operative venture of the Fisheries and Marine Service and the Environmental Protection Service of Environment Canada, the Nova Scotia Department of Fisheries and Alan Fisheries Limited of Digby, N. S. The report includes a brief review of depuration activities in the Maritimes and elsewhere; a detailed description of the pilot plant operations and equipment; details of the criteria and procedures used in the trials; an analysis of the results; and recommendations regarding operational procedures and equipment design. An analysis of the economics of depuration is contained in the appendices along with additional engineering design details, samples of recording materials, the raw bacteriological data and aspects of tank performance.

Depuration in the Maritimes and Elsewhere

In the past several years there has been an increased interest in the potential for shellfish depuration, both by the shellfish processing industry and fishermen, and by various federal and provincial fisheries agencies. Continued closure of shellfish producing areas due to municipal and local sewage contamination has reduced the availability of shellfish to the fisheries. The soft-shell clam fishery in particular has suffered, since simple relaying to clean water areas is less practical for this species than for others such as oysters. An accepted and approved method of "purifying" bacteriologically contaminated clams would enable the use of substantial stocks not presently available for harvest.

Bacteriologically contaminated molluscan shellfish (*e.g.* oysters, clams, mussels) will cleanse themselves when held under proper conditions in a controlled clean water environment, or when relayed to an area with sanitary and bacteriologically approved seawater. These two cleansing methods, "depuration" and "self-cleansing", are similar in that both involve the reduction to acceptable levels of contaminating micro-organisms in live shellfish. A "depuration" process uses a controlled environment with the shellfish held in baskets in tanks of flowing treated seawater. In "self-cleansing" the shellfish are held in floating trays or placed on the sea-bottom in the natural uncontrolled environment.

Neither controlled purification (depuration) nor self-cleansing have been used in the Maritimes since the early 1950's, the sole exception being the relaying of oysters from contaminated areas to approved seawater areas. Relaying for self-cleansing is also practiced in other areas for oysters and hard-shell clams, but not to our knowledge for soft-shell clams.

Between 1951 and 1953, experiments involving a semi-commercial procedure for the self-cleansing of soft-shell clams were carried out in Bocabec Bay, Charlotte County, N.B. (Mullan and Williams, 1954). In 1956-57, somewhat similar self-cleansing studies were conducted at St. Mary's Bay, P.E.I.; bay quahaugs *(Venus mercenaria)* were used (Swansburg and Mullan, 1957). These studies were conducted co-operatively by the Federal Department of Fisheries, the Fisheries Research Board and the Department of National Health and Welfare in conjunction with industry participants. Neither of these self-cleansing operations became commercial.

Depuration studies on oysters conducted on the West coast at Ladysmith, B. C. from 1969 to 1972 have been described by Devlin and Neufeld (1971) and Devlin (1973). Further studies on the depuration of several West coast clam species were conducted at the same facility in 1973 and 1974 (Neufeld et al, 1975).

On the East coast a semi-commercial soft-shell clam facility operated for a brief period in 1973 at St. Omer, Que., on the north shore of the Bay of Chaleur (Gaudet, 1973). The St. Omer project was not successful and was not continued beyond 1973. In the U. S., experiments on the depuration of shellfish have been conducted by a number of States, but only Maine, Massachusetts and New Jersey have had commercial depuration plants. A variety of shellfish are cleansed by different methods in several European countries such as Great Britain, France and Spain.

Controls and Regulations

During the 1950's, and throughout the 1960's, studies on controlled purification of shellfish were reported regularly to the National Shellfish Sanitation Workshops, sponsored by the Public Health Service of the U. S. Department of Health, Education and Welfare. Controlled purification gradually became referred to in the shellfish industry and in government agencies as "depuration". Furfari (1966) has summarized information on depuration for the National Shellfish Sanitation Program (NSSP).

The NSSP is a program administered by the U. S. Public Health Service, formulating voluntary and regulatory requirements for interstate shipments of live and processed (shucked) molluscan shellfish (except scallops). A bilateral agreement between Canada and the U.S.A. allows the certification of shellfish plants which enables export-import under the NSSP.

A Manual of Operations published by the NSSP serves as a procedural guideline for all matters pertaining to the public health in shellfish operations. In 1968, a "Proposed Part IV, Control of the Depuration Process", was presented to the 6th National Shellfish Sanitation Workshop, as an addition to the Manual of Operations (U.S. Dept. of Health, Education and Welfare, 1969). These procedural guidelines were further revised for presentation to the 7th National Shellfish Sanitation Workshop (Furfari, 1971).

Although the Manual of Operations provides the overall requirements for shellfish entering the U.S.A. market, including Canadian exports, shellfish processing operations in Canada, and their product, must as a first priority be acceptable under the regulations of the Federal Fish Inspection Act.

The control of a depuration plant requires protocols meeting the requirements of both the Manual of Operations and the Fish Inspection Act. This report should provide a basis for the establishment of such protocols.

During 1973, Regional Guidelines for Depuration of Shellfish were prepared by the Fisheries and Marine Service and the Environmental Protection Service of the Department of the Environment in response to inquiries from potential depuration plant operators and the Provincial Fisheries Departments of Nova Scotia and New Brunswick. These Regional Guidelines were prepared to identify needs and to establish a rational approach to the subject for intradepartmental administrative purposes.

Phase I of the Guidelines required that the extent of the softshell clam resource be determined for areas closed to harvesting for direct marketing but designated suitable for depuration. By the spring of 1974, clam population surveys had been completed in Charlotte County, N.B., and in the Annapolis Basin, N.S. These surveys indicated that sufficient clam resources were available to support commercial depuration and it was felt that Phase II involving a pilot plant operation was justified.

In 1974, a limited pilot plant operation was carried out at Alan Fisheries in Digby. Severe engineering problems related to the seawater intake system compressed the operational schedule and allowed only limited control of the depuration tank environment. Clams for the trials were harvested from widely separated areas of the Annapolis Basin in accordance with the original plan to determine if stocks from these areas could be successfully depurated. The majority of the nine lots processed did not depurate and it was impossible, because of the limited number of lots and erratic equipment function, to determine the cause(s). It is possible that operational and handling procedures may have produced thermal shock and/or physical disturbances which reduced the clams' ability to cleanse. Noise and vibration from other processing activity in the plant during treatment of several lots may also have been a contributing factor.

On review, it was felt that, with an improved intake and flow control system and better techniques in clam handling, depuration could be successfully conducted in the Annapolis Basin. The 1975 trials then proceeded as discussed in this report. To reduce geographical variability, only one small harvest area was used.

Participants

The project was planned by a Depuration Project Committee of the Maritimes Region Standing Committee on Shellfish. The Coordinator for Depuration Activities acted as Chairman of the Committee. The different agencies involved and their respective duties are listed below:

Fisheries and Marine Service

Resource Development Branch:

- Chairman
- Biological aspects of the depuration process
- Data analysis

Conservation and Protection Branch:

- Harvesting controls

Inspection Branch:

- Laboratory services
- Field co-ordination
- Technical services and engineering
- Advisor

Environmental Protection Service

Surveillance and Analysis Division:

- Laboratory facilities
- Advisor

Province of Nova Scotia

Department of Fisheries:

- Financial support and purchasing services
- Advisor

Alan Fisheries Limited

- Plant operator
- Advisor

Biological Considerations

In developing procedures for depuration it is necessary to consider the biology of the species to be depurated; species variability and seasonal factors relevant to depuration are among the factors to be considered with respect to the depuration of soft-shell clams in the Annapolis Basin.

Species Variability with Respect to Depuration

A tremendous variability exists in the physiological responses of various shellfish species to environmental factors and pollution. These points are emphasized in the works of Cabelli and Heffernan on the elimination of coliform bacteria by the soft-shell clam (1970) and the northern quahaug (1971).

Whether one wishes to depurate clams, quahaugs, oysters, or other species of shellfish, it should be kept in mind that the optimal range of environmental factors (temperature, salinity, etc.) established for one species does not necessarily apply to another. Depending on the species one is concerned with, accumulation and elimination of bacteria may proceed at different rates because of dissimilarities in feeding mechanisms, digestive processes, etc. To a lesser extent one should also take into account physiological variability within a species due to the establishment of separate populations of the species over a wide geographical range and the built-in animal-to-animal variability.

Taking these factors into consideration is important when attempts are made to set parameters to be used in determining acceptable harvest areas, depuration procedures and end product criteria.

Seasonal Factors Relevant to Depuration

Since this study deals with the depuration of soft-shell clams we will consider only those seasonal factors known to affect this particular species. The most well-documented of these factors is ambient water temperature. Cabelli and Heffernan (1970) examined the effect of temperatures from 2 to 16 C on the removal of bacteria by soft-shell clams. Inhibitory effects occurred when the water temperature was less than 8 C. While studying the growth of soft-shell clams in the Bay of Fundy area, Newcombe (1935a) determined that clams in this area would be most active at temperatures from 6 to 13 C. However, he (Newcombe, 1935b) adds along with Wilton and Wilton (1929) that the clam is quite sensitive to changing abundance in food supply and seasonal fluctuations in quantity of diatoms present. The minimum temperature for actively pumping clams may vary a few degrees between particular geographic locations.

Low temperatures have a marked effect on the microbial uptake by the clams. If the water temperature falls relatively low for an extended period of time, few coliform bacteria are present in the clams even though they are growing in polluted waters. Cabelli and Heffernan (1971) have shown the effect of seasonal changes on the presence of faecal coliforms in the northern quahaug. There is no reason to believe, even taking species variability into account, that soft-shell clams would behave much differently.

Other seasonal factors like salinity and oxygen concentrations were examined; they were considered not to have a significant effect in the physical environment of the Annapolis Basin.

EQUIPMENT DESIGN AND LAYOUT

This chapter presents a detailed description of the equipment used in the depuration trials; including specifications and design criteria, reasons for selection, and layout and function. Additional information may be found in Appendix B.

The facility, in an existing clam shucking plant, was located only four miles from the harvest area (Fig. 1).

Sea Water System

The seawater system consisted of the following components: submersible pump, intake line, strainers, filter, header tank, sterilizer, tank feed-pipes, flow-meters and controls, tanks and drains (Fig. 2).

Pump

The seawater source was located 1800 feet from shore to insure an adequate quantity of water of approved bacteriological quality at all phases of the tidal cycle. This location required the use of a submersible pump at the intake because of the long intake distance and lift (Fig. 1). The pump (Fig. 3) was sized to provide for a water flow of one gallon per minute per bushel for six tanks holding 21 bushels of clams and an additional capacity for washing. A 16 horsepower motor was required on the pump to develop an adequate pressure to overcome the combined pipe friction, filter and strainer pressure drop, and lift, while still providing sufficient discharge pressure. The pump motor required a 600-Volt, three-phase electrical source; this was located 2100 feet away at the main Digby wharf.

The pump (Fig. 3) was mounted centered in a steel screen box so that it would remain off bottom in any position. The pump intake was covered by a plastic screen.

Strainers

Seawater flowed from the pump through a buried three-inch diameter polyethylene pipe to the plant. Here the pipe was protected against bursting (due to possible accidental valve shut-off) by a pressure relief valve. On entering the plant, seawater could be passed through an in-series strainer and filter if conditions warranted filtration. These were by-passed during the 1975 trials since turbidity was very low. The water then flowed through a pipe to a flow control tank which delivered a constant flow to the U.V. sterilizer.

Sterilizer

The sterilizer, similar to the Kelly-Purdy type used in Maine (Sterl, 1964), was selected because it was inexpensive and easily cleaned and inspected (Fig. 4). The seawater flowed lengthwise through the sterilizer box over a corrugated bottom. The surface of the water was about twelve inches below the ten (30-watt) germicidal lamps. This was six inches greater than the recommended design, but subsequent testing indicated adequate performance. Readings of germicidal light intensity, measured after 500 hours of bulb use, varied from 500 watts per square centimeter at the center to 200 at the ends and sides with all ten lamps burning.

Flow-meter Box

The seawater flowed from the sterilizer through a header pipe to three flow-meter boxes; one per tank. The flow to each box was adjusted with a valve on the end of each pipe.

The water flowed into the flow-meter box (Fig. 5) and over a V-shaped weir, which gave a measure of flow-rate. This type of flow indicator was used because of its ease of construction and ease of cleaning (Furfari, 1966). Although automatic flow recorders were installed, missing and improper components delivered prevented their use. This necessitated regular monitoring of the V-notch weir.

Feed Pipe and Tank

After the water flowed over the weir, it travelled through a pipe (along one side of the tank) and then to the centre at the inlet end of the tank (Fig. 8). An elbow diverted the flow downward behind a baffle.

This initial arrangement of the water feed pipe behind the baffle was found to give an unbalanced flow of water between the sides of the tanks as indicated by the bacteriological results of trial 2. Dye tests conducted during trial 3 provided visual evidence of this imbalance.

A six-inch length of pipe attached to the elbow behind the baffle in tank 1 improved the distribution of the flow at the outlet end of the tank. Further details of the bacteriological and dye tests related to tank performance are given in Appendix E.

During depuration, the seawater flowed from under the baffle, around the clams, and out through three surface overflow pipes at the other end of the tank. These pipes carried the water to an open drainage trough in the floor which took water to a drain sump and out to the harbour.

During the tank washing operation, the water flowed from under the baffle, along the tank bottom, out the bottom drain hole and into the drainage trough.

Clam Processing Equipment

The clam processing equipment consisted of: a conveyor clam-washer, a basket filling tank, depuration baskets and tanks (Fig. 2).

Conveyor-washer

To reduce the amount of sediment and debris in the depuration tank, the clams were mechanically washed before being put into the depuration baskets. A spray-conveyor (similar to the type used in Maine) was selected to reduce labour costs and improve the efficiency of washing and culling (Fig. 6). This consisted of: a water bath, with overflow drain, to cushion the clams on loading; a screen-conveyor which extended from the bottom of the water bath through the sprays and culling sections to the basket filling tank; a rubber scraper blade over the conveyor; four sets of water sprays directed downward and two sets upward through the conveyor; two open inspection sections; and a discharge end.

Fresh water was fed into the bath through a hose, with excess water overflowing through a stand-pipe located under the baffle. For cleaning the bath, the baffle was removed and the stand-pipe was taken out to allow the sediment and debris to flush out through the drain. In operation, the baffle directed the clams to the conveyor. The angle of the conveyor and baffle were adjusted to aid in controlling the rate at which the clams were delivered to the sprays and culling stations. For cleaning, the end of the conveyor could be raised out of the bath. A rubber scraper blade over the conveyor was installed to maintain one layer of clams through the washer. This did not work well and it was necessary to spread the clams by hand. There were two culling stations on the washer; one in the receiving and washing room and one located in the tank room (Fig. 10). Each culling station could accommodate two cullers.

Basket Filling Tank

The conveyor discharged the clams to a tank filled with continuously flowing freshwater. This cushioned the fall of the clams into the depuration baskets and facilitated continuous loading of the baskets by allowing room for three baskets in the tank; one full and ready for removal, one filling and one empty.

Depuration Baskets

The depuration baskets were similar to those used in some plants in Maine, although slightly larger. The material was plastic (PVC) coated steel wire screen mesh (½" x ½") which allowed 71% open area for water flow (Fig. 7). The basket capacity, if filled, of 0.71 bushel (43 pounds) and the basket dimensions of 11" x 21⅝" x 6¾" were determined by the tank size and the width of the roll of screen mesh material. This design allowed for a clearance between the top of the clams and the top of the basket when filled to the operational capacity of 0.5 bushel. The plastic coating became abraded due to handling, but no rust was noted during the three months of trials. The baskets did not hold their shape well when loaded with clams.

Depuration Tanks

The tanks were sized to hold 21 bushels of clams in three layers of baskets (Fig. 8). The most readily available tank for this capacity was a white fiberglass reinforced tank of dimensions 4' x 8' x 3' deep. A baffle was installed at one end of the tank to distribute the water delivery evenly across the width of the tank; a similar design to the tanks used in Maine plants. The volume of the clam holding section of the tank (87" x 47" x 33" (water depth)) was 78.1 cubic feet. For maximum tank loading (21 bushels of clams) the volume ratio was 3.7 cubic feet per bushel, which was below the minimum suggested by Furfari (1966) of 5 cubic feet per bushel. The length/width ratio of 1.9 was just below the minimum of 2.0 suggested by Furfari (1966). The three layers of depuration baskets was one more than that used in Maine, but below the maximum of four suggested by Furfari (1966).

The tanks had flat bottoms but were sloped 1°16' (approx. ¼"/1') from the baffle end to the outlet end.

DEPURATION PROCEDURES AND CONTROL CRITERIA

This section outlines operational procedures, sampling techniques and procedures and control criteria used in the conduct of the depuration trials.

Harvesting Controls

The Conservation and Protection Branch of the Fisheries and Marine Service has responsibility for the enforcement of regulations controlling shellfish harvesting. This includes the control of harvesting for depuration. Harvesting and transport operations for the project were supervised by Fisheries Officers, with special attention being given to monitoring the handling and transport of the clams by the fishermen. This was considered particularly important since damage and shock can seriously affect subsequent depuration success. The experience gained and information accumulated during the project will provide a basis for the formulation of control procedures for future commercial operations.

Specific control measures included the issuance of a harvesting permit to each clam fisherman involved in the project. This permit was valid for harvesting from the Joggins area only. The fisherman was required to be in possession of his permit at all times while engaged in harvesting, landing or transporting shellstock from the harvest areas. The harvested shellfish were transported to the depuration plant in sealed enclosed trucks. Each trucker was issued a trucking permit. Sample copies of harvesting reports, harvesting permits, shellstock tags and seals used are presented in Appendix C of this report.

Criteria Used as Controls for Seawater and Shellstock

For use as food, contaminated shellfish must reduce their bacterial load sufficiently to comply with bacteriological standards which safeguard the public health. With depuration, physical and chemical criteria are used to insure that the shellfish may maintain a level of physiological activity adequate for "cleansing" in the controlled environment. The bacteriological standards as specified under the NSSP are the same regardless of the geographical location of a depuration facility whereas physical and chemical criteria may vary according to species and local differences.

Physical and Chemical Criteria on the Seawater

Seawater entering the depuration tank must be of a certain environmental quality. The most important factors are mentioned by Furfari (1971) in the proposed "Appendix D - Controlled Depuration" of the NSSP Manual of Operations.

They are:

Temperature	A minimum of 10 and maximum of 20 C but these may vary with species and geographical differences provided that pilot plant studies demonstrate it.[1]
Turbidity	Maximum of 20 Jackson Turbidity Units.
pH	From 7.0 to 8.4.
Salinity	Within 20% of the value of the harvest area water expressed in parts per thousand.
Dissolved Oxygen	Minimum of 5.0 mg/l.
Heavy metals, organics and radioisotopes	Level low enough that the shellfish activity will not be affected and subsequent levels in depurated shellfish will not exceed acceptable standards.
Paralytic Shellfish Poisoning	Not present in quantities which would be concentrated by shellfish to a level exceeding 80 micrograms per 100 grams.

Bacteriological Criteria

Seawater: Seawater to be used for depuration purposes must be of sufficiently good quality that U.V. treatment will consistently reduce the bacterial count to <2 total coliform MPN per 100 ml of water.

Shellstock for Depuration: Areas for shellfish harvesting are identified on the basis of the faecal coliform MPN count in the overlay water. An area is closed on account of domestic pollution when sampling for faecal coliform reveals a median value of 14 MPN and 10% of the samples exceeding 43 MPN per 100 ml of water for a 5-tube multiple dilution test (total coliform equivalent: median 70 MPN and 10% over 230 MPN). Moderately contaminated areas, having a total coliform of 231 to 700 MPN in the overlay waters, may be designated for harvesting for depuration. At times there may be considerable variation between the MPN count actually found in the shellstock and what was expected on the basis of overlay water sampling. Cook (1974) reports limited evidence to this effect based on the 1974 depuration trials. This variability can be highly significant in commercial depuration where the initial bacterial load of the shellstock is used as an indicator of potential for positive depuration.

If possible, the bacterial load of shellstock from each harvesting area designated for depuration should be determined. In this project, a limited number of shellstock samples were analyzed. A further discussion is to be found in the Depuration Trials section.

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[1] Furfari (1966) gives a temperature range of 2-20 C for the depuration of soft-shell clams.

Depurated Shellstock: Shellstock is considered depurated when the faecal coliform load has been reduced to a level considered acceptable to the protection of the public health. The criteria used are based on the premise that the process and plant involved have demonstrated reliability. This is not the case in a pilot operation such as this.

For the purpose of releasing a "depurated" lot during these trials, the acceptable level was determined on a single value, the maximum faecal coliform count obtained from the 6 bacteriological samples analyzed for each lot.

A maximum faecal coliform count of 50 MPN or less in a lot was considered totally satisfactory.

A maximum faecal coliform count between 50 and 230 MPN in a lot was considered less satisfactory but acceptable for release.

A maximum faecal coliform count exceeding 230 MPN in a lot meant rejection of that particular lot.

These criteria for depurated shellfish were applied on a lot to lot basis and were based on a single count (maximum) out of a number of samples per lot. These restrictive criteria were felt necessary because of the experimental nature of the depuration trials. Since the purpose of the trials was to evaluate factors whose effects were not known, operational procedures had to be continually varied. The standard criteria used in proven commercial operations could not be effectively applied under these experimental conditions.

Depuration as a routine commercial operation does not require such stringent control measures. Variability is much reduced since the same operational procedures take place over and over again. Criteria of performance, applied over a period of time, are then a more appropriate means of assessing depuration. To perform the over-all assessment of the depuration trials the criteria of performance recommended by the 7th National Shellfish Sanitation Workshop (Proc. 7th National Shellfish Sanitation Workshop, 1971) were used. "The standard would be for routine operations with a median value of samples of depurated soft-shell clams not exceeding a faecal coliform MPN of 50 per 100 grams of sample and not more than 10 percent of the samples should exceed a faecal coliform MPN of 130 per 100 grams of sample."

The Most Probable Number (MPN) technique is extensively used in food microbiology to obtain an estimate of bacterial concentrations. This technique is based on probability statistics and results are directly related to the frequency of occurrence of a series of positive tubes most likely to occur. On a probability scale the median value represents the mid-point value of the results. Confidence limits may also be applied to the results obtained. Oblinger and Koburger (1975) in a general discussion of the MPN technique quote Woodward (1957) that the 95% confidence limits cover a 13-fold range, from approximately 24 to 324% of the MPN count in a five-tube multiple dilution test. One must be aware of the limitations of the MPN test in order to accurately interpret bacteriological counts resulting from the analysis.

Sampling Methods

Physical and chemical factors including temperature, salinity, turbidity, dissolved oxygen, and pH were recorded once daily when a depuration trial was taking place. Most readings were performed on both the raw and the U.V. treated seawater. Only values from the treated water will be presented in this report. These values were highly similar to the values obtained for the raw water.

Temperature was measured with a mercury glass thermometer.

Salinity was measured by the Knudsen method.

Turbidity was determined by colorimetry in Jackson Turbidity Units, JTU (color filter 4445, Hach Kit, Hach Chemical Co., Ames, Iowa, U.S.A.).

Dissolved oxygen was determined by the modified Azide-Winkler method with drop count titration (Hach Kit).

pH was measured using pH Alkacid paper, short range (6-8.5), (Fisher Scientific Co.).

Bacteriological assays were carried out on water and clam samples. Samples were analyzed within a short period of time after collection, the mobile laboratory being located 100 feet from the depuration plant. The enumeration of faecal coliform bacteria was performed through the Most Probable Number (MPN) method. The five-tube multiple dilution technique was utilized as specified in Part 3 of the manual "Standard Procedures for Bacteriological Analysis" (Inspection Branch, Fisheries and Marine Service, 1972).

For each trial samples of the raw seawater, the U.V. treated water, and the water at the effluent outlets of each depuration tank were collected at 0 Hour and at the 18 or 24 Hour and the 42 or 48 Hour clean-up period.

Six samples of clams per tank were usually collected to determine bacterial level at 0 Hour, 18-24 Hour, and 42-48 Hour. Each sample consisted of a composite of 10 to 12 clams taken from the top of stacked baskets. If the tank was loaded with 30, 36 or 42 baskets, 3 baskets would constitute a stack. If the tank was loaded with 28 baskets, the stack would consist of 2 baskets. Clam samples were collected in plastic bags for transport to the laboratory, and stored in a refrigerator prior to analysis.

Plant Operations

The depuration plant operations are illustrated in Figures 9 and 10. The clam washing and raw seawater treatment took place in a room separate from the tank room in order to reduce chances of recontamination of the clams and to conform to Inspection Regulations.

The seawater system was started as soon as the clams to be depurated arrived at the plant. The U.V. sterilizer was also turned on and the sterile water was allowed to flow through the feed pipes into the depuration tanks and out the open drains. This allowed a thorough flushing of the system prior to depuration.

The clams were taken from the diggers' trucks and placed on the floor near the conveyor-washer. They were then emptied from the onion bags into the water bath and conveyed through the spray and culling sections after which they dropped into a depuration basket submerged in the filling tank. After a basket was filled to a depth of approximately five inches (28 to 33 pounds of clams) it was slid sideways and an empty basket was moved into its place at the end of the conveyor. The loaded basket was then removed and placed on the weigh scale. The baskets were then stacked in three separate piles so that the weight of clams in each pile could be adjusted to the required amount for each tank. The numbers of bushels for each tank was calculated on the basis of sixty pounds of clams per bushel. After the correct amount of clams were distributed to each pile, the spacer pipes were placed on the tank bottom and the depuration baskets were placed on the spacers according to the desired arrangement. Spacers were used between each subsequent layer of baskets. The tank drain was then closed to allow the treated water to fill the tank. While the tank was filling, the seawater flow-rate was adjusted by the valve over the flow-meter box to give the desired flow for the number of bushels in the tank.

Timing for the various operational steps of the process was established to coincide with the tides in order to insure that the pumping activity of the clams was maintained at an optimum level. This timing was considered desirable since *Mya arenaria* is known to be highly susceptible to physiological stress (Cabelli and Heffernan, 1970).

The extreme amplitude of the tides in the Annapolis Basin and the likelihood of a tidal linked bio-rhythm in the clams was a further reason for considering that pumping activity and bacterial reduction might be optimised by such timing.

The pumping rate of clams is related primarily to temperature; a direct correlation existing between rate of metabolism and temperature. It may also be related to the amount of food particles available in the surrounding water (Langton, 1975; Newcombe, 1935b). Because of the oceanographic characteristics of the Annapolis Basin area, mainly the amplitude of the tidal flushing, the relative abundance of the food supply almost certainly varies in a cyclic fashion with the tides.

Evidence of tidal related bio-rhythms has been recorded for many littoral bivalves (Morton, 1956; Morton, 1970; Owen, 1972). Even though there is less specific evidence on the rhythmicity of *Mya* (Morton, 1973) than of other littoral bivalves, it is worthwhile to consider this phenomenon, especially as related to the dynamics of the digestion process. According to these studies respiration, ingestion, and expulsion of faeces and pseudofaeces occur almost exclusively during submergence (high tide) while digestion takes place during exposure (low tide).

The operational methodology and the timing of the depuration process relative to the tidal cycle was initially as follows: The "0" Hour of the depuration process (when the water first started to flow out the overflow pipes) corresponded to the first high tide following the low tide period when the clams had been dug. The flow-rate was maintained for the time required for the first stage of cleansing (24 hours from 0 Hour or in other words, until the high tide of the next day). At this time the tank was drained and the baskets of clams removed and placed on the floor for hosing down. Bacteriological sampling was performed. The clams were washed in the baskets with a low pressure flow of freshwater from a hand held hose. The tanks were similarly washed and scrubbed with a soft bristled brush. The debris were washed out of the tank bottom drain. The hollow spacer pipes were individually hosed out. The tanks were then reloaded and filled with seawater for the second 24-hour depuration stage.

After trial 2, it was noticed that draining the tank and exposing the clams at the high tide point, when they had been pumping most actively, was considerably slowing down the "recovery process". After the tank was filled the clams would not resume pumping for several hours. This is to be expected if they follow any kind of tidal rhythmicity; pumping rate is slowing down as the tidal cycle goes into the ebbing phase. It was then decided to drain the tank for bacteriological sampling and cleaning up at the low tide point, making it "18" and "42" Hour. In this instance, clams were observed to resume pumping as soon as covered by water; the tide going into the flooding phase at this time.

At the end of the second stage (42 Hour) the clams were removed from the tanks, hosed down, sampled for bacteriological purposes and put into perforated plastic boxes for storage in a refrigerated room (held at 3.3 C). They were then held until results of the bacteriological sampling were received.

DEPURATION TRIALS

Bacteriological results obtained from the limited trials conducted in 1974 indicated that a step by step procedure that would ensure satisfactory depuration could not be provided without additional study. These results further indicated that many of the recommended requirements in Appendix D of the Manual of Operations may not be directly applicable in an area such as the Annapolis Basin.

It was felt that the 1975 project should be directed at the development of an operationally reliable physical plant and the determination of the operational, physical, chemical and bacteriological parameters necessary for successful commercial scale depuration in the Annapolis Basin. This required a complete redesign and expansion of the physical plant to a semi-commercial scale.

Constraints imposed by the semi-commercial scale of the project, limited laboratory capacity for handling the volume of bacteriological sampling and testing required by the experiments, and general time and budget restrictions all were important in determining the extent of studies possible. As the trials proceeded, the results of each trial were taken into consideration and the planned schedule modified to make more effective use of the time available.

This chapter outlines the purpose of each of the trials conducted, variations in the operational methodology for each experimental lot, and results.

During each trial, tests were conducted to monitor physical, chemical and bacteriological factors of both the raw and U.V. treated seawater. The results of these tests are given in Tables 1 and 2 along with a record of the tidal phase at time of sampling.

Because turbidity levels were below the sensitivity of the test the seawater filtering system was not used. The temperature of the treated seawater entering the depuration tanks ranged from 8.2 to 12.9 C. This is well above the 2 C minimum recommended for *Mya* (Furfari, 1966), and within the 6 - 13.5 C range at which *Mya* is most active in this region (Newcombe, 1935a). Salinity of the process water ranged from 24.6 to 33.7‰. This is probably slightly higher than that of the overlay water in the harvest area but within the 20% limit recommended (Furfari, 1966). Dissolved oxygen varied from 8 to 10 mg/l and was well above the 5 mg/l recommended as a minimum. Hydrogen ion concentration (pH) was recorded but the results were found to be inaccurate and no conclusions are possible.

Bacteriological tests on the raw seawater, prior to U.V. treatment, gave a range of <2-130 MPN faecal coliform (F.C.)/100 ml indicating very good quality water. U.V. treatment of the process water reduced F.C. levels to <2 MPN. At the start of trial 2 the water sampled as "treated" had in fact not been treated since the

U.V. system was not operating, hence the high counts shown at the top of Table 2. The bacterial counts of the effluent from the depuration tanks ranged from 2 to 8 MPN F.C./100 ml indicating only a small removal of faecal coliform from the tank during cleansing.

An outline of the nine trials conducted follows. The tables (Tables 3 to 11) summarize the bacteriological results of each trial, but for greater detail the reader must refer to Appendix D.

Because of tank and time limitations it was sometimes necessary to examine more than one variable in a particular trial. This was often further complicated by the variability in 0 Hour F.C. count between the clam stock placed in the various tanks. This 0 Hour variability persisted even though the stocks were harvested from only one small area. Due to the nature of the bacteriological tests the 0 Hour counts are not available until more than 48 hours after the samples are taken. These limitations made it necessary, after the initial investigation of any variable, to carry out one or two confirmations in subsequent trials.

Trial Objectives, Procedures and Results

Trial 1

The purpose of this trial was to determine the optimum seawater flow-rate for depuration of a fully loaded tank with 3 layers of 14 baskets (42 baskets in total). Flow-rates of 0.5, 0.7 and 1.0 gpm/bu were established in three separate tanks. All tanks were drained and hosed down at 24 Hour, but the baskets were not hosed down.

The results (Table 3) indicate better cleansing activity with flow-rates of 0.5 and 0.7 gpm/bu than at 1.0 gpm/bu.

Trial 2

The purpose of this trial was to determine the uniformity of depuration throughout the tank and to follow cleansing activity beyond the standard 48-hour period. The tank, loaded with 42 baskets, was drained at 24, 48 and 72 Hour. Based on the results of trial 1, a flow rate of 0.5 gpm/bu was used. The baskets of clams were removed after draining, the tank was hosed down, but the baskets were not hosed down.

Table 4 indicates only the bacteriological pattern throughout the 72-hour depuration cycle. Results indicate good depuration within 24 hours with insignificant increases in bacteriological load at 48 and 72 Hour. Results on uniformity of depuration throughout the tank require extensive discussion and are presented in Appendix E.

Trial 3

Beginning with this trial, draining, hosing down and bacteriological sampling were changed to 18 and 42 Hour as discussed in the

Plant Operations section. The purposes of this trial were twofold: 1) to confirm that 0.5 gpm/bu, as suggested from trial 1 and 2, was as satisfactory as a higher flow-rate of 1.0 gpm/bu; and 2) to determine the effect of hosing down the tanks and clam baskets after tank draining.

The trial was set up in such a way that the effect of flow-rate could be determined from tanks 2 and 3 and the effects of hosing from tanks 1 and 2.

The results (Table 5) of tanks 2 and 3 indicate no significant difference between flow-rates of 0.5 gpm/bu and 1.0 gpm/bu. The results of tanks 1 and 2 indicate that hosing down of the tank and baskets is important to the success of depuration.

Trial 4

The purpose of this trial was to further confirm the results of trials 1 and 3 on flow-rate and the results of trial 3 on the hosing down of the tank and baskets.

The results (Table 6) indicate better depuration up to 18 hours for the 0.5 gpm/bu flow-rate. This in spite of a much higher initial bacterial loading in the clams of tanks 1 and 2. Because of the much higher bacterial load of the clams used in this trial it is impossible to relate the results to those of trial 3 and no conclusions can be reached regarding the effectiveness of hosing down.

Trial 5

The purpose of this trial was to determine the effects of reduced loading of baskets in the tanks and prolonged exposure to simulate a low tide midway through the depuration cycle.

Tanks 1 and 2 had 3 layers of 12 baskets (36 baskets total) and tank 3 had 3 layers of 14 baskets (42 baskets total).

As indicated by Table 7, the 0 Hour bacterial loads were extremely high and the trial could not fulfill its purpose.

Trial 6

The purpose of this trial was to determine the effect of increasing the available tank volume for water circulation between the sides of the baskets. This was achieved by reducing the number of baskets to 3 layers of 10 (30 baskets total). A second purpose was to study the effect of not draining the tank midway through the depuration cycle at 18 Hour. It was felt that not draining at 18 Hour would cause less disturbance to the clams and would reduce the fragmentation of bacterial clumps in the tank. This fragmentation has been suggested (Hurst, personal communication) as a possible cause of recontamination during the latter stages of the depuration cycle. Not draining precluded hosing down and removal of waste materials from both the tank and baskets.

Due to problems in availability of clam diggers an insufficient volume of clams was obtained and the experiment could not be conducted as planned. It was decided, however, to run one tank at the reduced loading and with no drainage at 18 Hour.

The results (Table 8) indicate a negative effect to an increased water volume between the sides of the baskets. There is no indication in the results as to the effect of not draining at 18 Hour.

Trial 7

The purpose of this trial was to determine the effect of increasing the available tank volume for water circulation at the bottom of the tank. This was achieved by placing a layer of empty inverted baskets in the bottom of tanks 2 and 3 and reducing the tank loading to 2 layers of 14 baskets (28 baskets total). A second purpose was to study the effect of thorough disinfection of all equipment prior to depuration.

The effect of increasing water volume at the bottom of the tank can be determined from a comparison of the results of tanks 1 and 2. The effect of thorough disinfection can be determined from comparison of tanks 2 and 3.

The results (Table 9) indicate that an increase in water volume at the bottom of the tanks improved depuration. There is no indication that thorough disinfection benefited the depuration process.

Trial 8

The purpose of this trial was to determine the effect of additional waste (faeces, pseudofaeces and debris) removal from the tank through increased hosing down. Two alternate methods were used. While tank 1 was used as the standard, tank 2 was given an additional drainage and hosing down at 30 Hour and tank 3 was given a more complete hosing down of the clam baskets at the regular 18 Hour drainage. In the case of tank 2, the 30 Hour drainage point was selected to correspond with the next low tide period after 18 Hour. For tank 3, the more complete hosing down consisted of emptying the clams from the baskets, spreading them out in larger baskets, hosing them thoroughly and replacing them in their original baskets. These baskets were also thoroughly hosed while empty.

For this trial the 28 baskets configuration was used in all tanks.

Although tanks 2 and 3 gave better end results than tank 1, the bacteriological sampling at 30 Hour tends to show that fluctuations in the bacterial load of the clams may be quite important over a short period of time in the tank environment. The representativeness of the clams sampled and the variability of the MPN test used may tend to amplify these apparent fluctuations.

The results (Table 10) suggest that additional waste removal through draining and hosing promotes more complete depuration.

Trial 9

This trial was designed to duplicate trial 8 and to follow the changes in bacterial load during the latter stages of depuration. Additional bacteriological sampling was carried out at 28, 32, and 40 Hour.

Because of the high bacterial load in the clams of tank 2 at 0 Hour, the results (Table 11) from this tank cannot be used to determine the effectiveness of additional waste removal.

A comparison of the results of tanks 1 and 3 appear to indicate that additional waste removal is beneficial to depuration success.

Analysis and Discussion of Results

To discuss the results of the depuration trials further, the experimental lots have been divided into three groups according to the bacterial counts:

(1) Experimental lots with an end product meeting the established criteria for depurated shellfish. i.e., the 42 Hour bacteriological count shows thorough depuration;

(2) Experimental lots with an end product not meeting the established criteria for depurated shellfish but having an initial bacterial load comparable to the loads in (1) above;

(3) Experimental lots with an end product not meeting the established criteria for depurated shellfish and having an initial bacterial load greater than in (1) and (2) above.

A few related items are also presented.

Group 1, Experimental Lots With an End Product Meeting the Established Criteria for Depurated Shellfish

The criteria used to determine if an experimental lot has depurated thoroughly is the one recommended under the NSSP for routine depuration operations. This standard is outlined in an earlier section of this report (page 12).

Table 12 lists those lots which depurated thoroughly. Each lot is identified by a "Trial-Tank" pair of numbers. The performance of a lot is assessed on the basis of the median and maximum 42-48 Hour counts derived from the 6 samples taken per lot. To be included in this group each lot should have 3 samples out of 6 (50% or median) with a count of 50 MPN or less and a maximum count of 130 MPN or less. In addition, 2 lots out of the total 23 (10%) experimental lots (lots 1-2 and 9-3) are included in this group. They fulfill the first requirement of a median count of 50 MPN or less. The maximum counts of these 2 lots exceed 130 MPN but they are considered as being the "10% of the samples exceeding 130 MPN F.C. per 100 g".

These two lots with a median count of 50 MPN or less have the next lowest maximum counts of all the experimental lots. All these lots have reduced their initial bacterial load significantly, from a 0 Hour median count of 490 MPN F.C./100 g to a 42-48 Hour median count of 20 MPN F.C./100 g (Table 13). The range of all MPN counts for 0 Hour should be noted (130-2200 MPN F.C./100 g). Initial bacterial loading is an important factor when considering bacterial reduction to a very low level. The results are illustrated graphically as probability plots (Fig. 11). Figure 12 shows the reduction of bacteria during the depuration cycle. In two cases, lots 8-3 and 9-3 there is a slight increase in faecal coliforms, the phenomena is called "bouncing", from the 18-24 Hour sample count to the 42-48 Hour sample count. However, these are not significant.

Attempts were made to correlate successful depuration with factors relevant to the operational methodology once the clam shellstocks have been placed in the depuration tank (cf. Table 12). The coupled effects of drainage and hosing of the tank, and hosing of the holding baskets at the 18-24 Hour are certainly contributing to depuration. In the case of lots 8-2, 8-3 and 9-3, hosing was emphasized by a 30 Hour hosing down or by more complete hosing down of the baskets at 18 Hour. This is shown to be beneficial but not essential. The results also indicate that a flow-rate of 0.5 gpm per bushel is reliable. Apparently, the load of clams placed in a depuration tank did not matter to any extent. The distribution of the baskets in 2 or 3 layers did not seem to have any importance either.

Examining the results of these experimental lots reveals that initial bacterial loading within a limited range and certain operation procedures (flow-rate, drainage and hosing), are the main factors leading to positive depuration provided that the treated seawater is of good environmental quality.

Group 2, Experimental Lots With an End Product not Meeting the Criteria for Depurated Shellfish Yet Having an Initial Bacterial Load Comparable to the Loads in Group 1.

Table 14 presents the next series of experimental lots. The initial bacterial loadings have a range of 80 to 1300 MPN F.C. per 100 g with a median value of 460 MPN (Table 15). The 0 Hour median value is similar to the one of lots which depurated successfully (Table 13). There is little variability between the 0 Hour standard deviation of the two groups of lots. From 0 Hour to 18-24 hours of depuration time these lots decreased their initial loading markedly (median 460 to 120 MPN, Table 15 and Fig. 13), except in two cases, 6-3 and 7-1. However, once the clams got rid of the main load of bacteria from the beach in the first 18-24 hours of depuration they have increased it again as shown by the 42-48 Hour bacteriological sample (median 220 MPN; range <20-24000+). The probability plots (Fig. 14) illustrate the variability of the results especially at 42-48 Hour.

A detailed examination of these lots and how they were treated may provide explanations on the final bacteriological results. Lot 1-3 decreased its bacterial loading to an acceptable level before bouncing up again. The flow-rate was maintained at 1 gpm/bu and no hosing of the holding baskets took place (Table 14). If lots were admitted in the group of experimental lots with a depurated end product on the basis of the 42-48 Hour median count only lot 2-1 would belong to Group 1 above. The 42-48 Hour median value of lot 2-1 is 35 MPN. One high count (700 MPN) out of the 6 samples justified its inclusion in Group 2. If basket hosing would have been performed it might have eliminated any high count present. In the case of lot 3-1 there was no hosing of the tank or holding baskets. The flow-rate was 1 gpm/bu. A combination of these unideal conditions may have led to the slight increase in faecal coliforms. Lot 6-3 bacteriological sampling shows a steady increase of bacteria in the clams as depuration was taking place. For this particular lot, baskets were disposed in 3 layers of 10 baskets each with plenty of space all around them. There was no drainage or hosing of any kind throughout the whole depuration cycle. The median values of the three bacteriological samplings, 0 Hour, 18-24 Hour, and 42-48 Hour of lot 7-1 stayed almost the same through the whole depuration cycle. Nothing in the procedures could explain this steady state. The median values of the 42-48 Hour sample of lot 7-3 and lot 9-1 show a slight increase in faecal coliforms from 18-42 Hours (lot 7-3: 65 to 130 MPN; lot 9-1: 90 to 150 MPN). This increase is not too significant as far as MPN are concerned and may not be attributed to faulty procedures.

The examination of these lots' results tends to indicate the importance of a good flow-rate, drainage and hosing. Despite these procedures however, a few lots did not depurate sufficiently to meet the criteria set for depurated shellfish. Lots 7-3 and 9-1 did decrease their bacterial load; the slight 42-48 Hour pick-up is not highly significant. Only the lack of depuration of lot 7-1 remains unexplained in terms of operating procedures.

During the first 18-24 hours of depuration the clams get rid of the main bacterial load. Elimination of the remaining faecal coliforms depends on operational procedures and equipment function. Attention has been brought earlier to the operational aspect. Equipment-wise, the U.V. sterilizer and the depuration tank, the two most important pieces of equipment, should have a high degree of performance. The U.V. sterilizer should provide treated water of sterile quality. Examination of water samples at the outlet end of the unit proves that it did. It is also very important to have a uniform distribution of the water flow in any depuration tank. This, we shall call tank performance. It should be noted that none of the lots dealt with in Group 2 were processed in tank 2; there were 4 lots from tank 1 and 3 lots from tank 3. Four of the 8 lots in Group 1 were processed in tank 2. This difference in depuration success could be due in part to small individual differences among the tanks. We may suppose that "bouncing" is more likely to happen in those tanks where flow distribution is less uniform. The water circulation pattern and performance of a particular tank may vary considerably depending on its design, its contents and their distribution. Tank performance is examined in Appendix E.

Group 3, Experimental Lots Which Did Not Reduce Their Bacterial Load to Meet the Criteria Set for Depurated Shellfish and Which Had Initial Bacterial Loads Greater Than Groups 1 and 2

The lots included in the Group (Table 16) have initial bacterial loadings approximately 10 times greater than the loading of the previous groups. During the first 18 or 24 hours of depuration time, a portion of these lots decreased their faecal coliform count quite significantly (Fig. 15). However, the broken line indicates that the coliform counts plotted here belong to two different statistical populations of counts; one population that depurated to a limited extent and another whose coliform level did not decrease significantly. By the final sampling time at 42-48 Hour all resulting counts belong to one population of counts again. Counts are as high as they were initially. Cabelli and Heffernan (1970) have demonstrated experimentally that soft-shell clams having high initial bacterial loads will have high residual loads after the normal 48 hour depuration period. In this relationship the residual loads are highly variable, although of the same order of magnitude as the initial loads. Piel, Ceurvels, DerHovanesian and Pow (1974) have demonstrated these same phenomena statistically. The results for Group 3, as illustrated in Fig. 15 and 16, are in accord with these findings.

The "bouncing phenomenon" observed between 18-24 Hour and 42-48 Hour (Fig. 13 and 16) appears to be amplified by a greater number of faecal coliforms in the initial loading.

Briefly, our attempts to depurate lots with high initial bacterial loadings (median value 4300 MPN) to meet the criteria for depurated shellfish within a limited period of time (42 hours) did not encounter any success. Table 16 attests that the procedures used were of the same nature as those used for lots that depurated successfully. These results tend to suggest that initial bacterial loading is a limiting factor to complete depuration within a reasonable period of time.

Related Items

Biochemical Identification of Faecal Coliform Isolates: When bacteriological sampling revealed the presence of very high faecal coliform counts (24000 MPN), starting with trial 4, laboratory procedures were reviewed to confirm standard practice. Possible contamination sources in the physical plant were also looked at as possible causes for the tremendous increase over the previous trials. A careful check of the laboratory procedures employed allowed rejection of the hypothesis that they were the source of the elevated counts. To examine possible contamination at the plant, biochemical identification of faecal coliform isolates was performed on certain lots. Identification was first carried out on the same clam meat samples used to perform the MPN test for faecal coliforms. Then the same biochemical tests were done on swab samples from different locations of the depuration tank. On two occasions, trials 5 and 6 (Tables 17 and 18), biochemical tests of clam meat samples identify *Escherichia coli* as being only 35 to 45% of the faecal coliforms present at the 0 Hour sampling time. By the 42 Hour sampling time, *E. coli* presence is reduced to 30 and 12% respectively. While depuration is going on, Klebsiella types of bacteria are steadily increasing. This may be explained without the intervention of external contamination as causal agent. Klebsiella types of bacteria thrive very well in a slimy environment. The clams, densely stacked in the depuration tank, eject their faeces as mucus strings which accumulate in the tank creating ideal conditions for Klebsiella types of bacteria. The problem may become more acute when bacteria levels are high to start with. The portions of Enterobacter and Citrobacter types of bacteria did not fluctuate to the extent of the *E. coli* and Klebsiella types of bacteria fractions. Proportionwise, the biochemical tests indicate a shift from *E. coli* to Klebsiella types of bacteria during the 42 hours of depuration. Attention should be brought here to the lack of specificity of the faecal coliform test. *E. coli* is of main concern as an indicator of more severe pathogens yet the faecal coliform test does not adequately represent *E. coli*. The test does not distinguish between *E. coli*, Klebsiella, Enterobacter and Citrobacter types of bacteria. Bacteriological techniques as they stand at present do not provide a test that would be more specific to *E. coli* yet as reliable as the faecal coliform test.

Swab samples from the shell surface of the clam, from the bottom of the wire mesh depuration basket, and from the bottom of the depuration tank (Table 19) before and after hosing down seem to indicate that the clean-up procedures do a good job at getting rid of bacteria. After hosing down, MPN tests show a minimum level of coliforms based on the sensitivity of the test. They eliminate the possibility of contamination or reinoculation.

Faecal Coliform Counts in Clams Prior to Depuration: Faecal coliform MPN tests were also performed on some samples of clams coming directly from the beach (shellstock samples) on certain dates during the summer (Table 20). The faecal coliform counts of these samples were pooled with the 0 Hour counts of the depuration trials (clams sampled upon arrival at the fish plant) to study the distribution of counts on probability paper (Fig. 17). The plotting of the counts reveals two statistical populations of counts indicated by the broken line. The 2000 MPN count may approximately divide the two populations. No count belonging to the "lower counts population" is higher than 2000 MPN. Table 21 gives the median MPN values for portions of the two statistical populations derived from shellstock and 0 Hour counts. At first the counts of the shellstock are considered separately from the 0 Hour counts. Then median values are given for the shellstock counts and the 0 Hour counts reassembled. From a total number of samples of 125 the median value of the "below 2000 MPN population" is 490 MPN. The "above 2000 MPN population" has a median value of 9200 MPN. Referring to what has been established previously, it should be possible to depurate the "below 2000 MPN population" so that its end product meets the criteria set for depurated shellfish within a reasonable period of time. However, it is highly doubtful that the same goal could be attained within the same time period with the "above 2000 MPN population".

What factor(s) may be responsible for the split of counts into two different populations? All clams dug for the experimental trials were comming from one particular location of the Joggins area, a total harvested area of 20,000 square yards. Environmental conditions within this area were quite uniform eliminating the possibility of a spatial variability. A temporal element was also looked at as a dissociation factor. Figure 18 illustrates faecal coliform counts versus date of sampling during the summer. The "above 2000 MPN population" is composed of counts occurring mainly during the month of July, samples of the 9th, 15th, 23rd and 30th. A few other counts belonging to this population also show up during August, but they are of minor importance compared to the July occurrences. The "below 2000 MPN population" includes counts spread over the whole experimental period but mainly before and after the month of July. It appears the harvest area used is subject to an increase in faecal coliform levels during part of the summer.

A bacteriological study of the Annapolis Basin area performed by the Environmental Protection Service of Environment Canada (Menon, 1974) had reported an increase of faecal coliforms in the overlay waters of the shellfish growing areas during the summer. However, their sampling program did not allow for close monitoring of the fluctuations of faecal coliform levels in the shellfish. The tem-poral increase of faecal coliform counts during the month of July may be related to increased "coliform activity" of neighboring areas (due to tourism) as well as higher summer temperatures. This analysis suggests the necessity of close monitoring of shellfish growing areas "approved for depuration". Further experience may show that these can be "approved for depuration" only on a seasonal or temporary basis. The extreme amplitude of the tides in the Annapolis Basin area may add one extra dimension to the circulation pattern of bacteria in the overlay waters. Pockets of highly contaminated water may exist for a period of time before becoming diluted to below the limit considered acceptable for open areas or being flushed out of the Basin.

From the point of view of both the regulatory authorities and the clam industry, it becomes an important asset to have knowledge of the bacterial loading of the shellstock being harvested. It enables the clam fisherman to decide, depending on the location and time, whether digging is worthwhile. In depuration early knowledge of the initial loading on a lot-by-lot basis is also important. The bacteriological techniques presently used require 48 hours to establish what the initial bacterial loading is. By the time the loading is known the particular lot has been through the complete depuration cycle whether or not it was a worthwhile undertaking. Improved bacteriological sampling techniques such as the Elevated Temperature Coliform Plate Count (Heffernan and Cabelli, 1967) allow determination of bacterial loading within 24 hours. Future depuration operations will undoubtedly use this or other more rapid techniques.

CONCLUSIONS AND RECOMMENDATIONS

This semi-commercial scale study has demonstrated that soft-shell clams (*Mya arenaria*) from moderately contaminated areas of the Annapolis Basin can be successfully depurated if proper operational criteria and procedures are maintained. Generally, shellstock having a faecal coliform count of <2000 MPN can be depurated within 42 hours, provided a flow-rate of 0.5 gpm per bushel is maintained and the tanks, baskets, spacers and clams are thoroughly hosed down at the mid-cycle (18 Hour) drainage period. Based on these results, it is recommended that controlled commercial depuration should be permitted in the Digby plant of Alan Fisheries Limited. Initial operations should be closely monitored until good reliability has been demonstrated over a period of time.

Problems associated with the development of high F.C. MPN counts in the shellstock during periods of the summer, be they due to *E. coli* or the probably harmless citrate-utilizing bacteria, may restrict commercial operations to particular seasons or areas of the Basin. These problems will be more clearly defined only with regular data accumulation from a reliable commercial operation.

The quality of the seawater available at the Digby plant is fully adequate. No treatment, other than passage through the U.V. system, appears necessary.

For ease of reference, recommendations arising from this study have been divided into three categories. They are: "Operational", if related to the day-to-day operational criteria and procedures; "Equipment Design and Function", if related to structural, material or functional aspects affecting the efficiency of the operation; and "General", if related to aspects of importance not only to this particular operation, but to depuration in general.

Operational

1. Shellstock to be depurated should not have a bacterial load exceeding 1600 MPN faecal coliform per 100 g of meat. While this is below the 2000 MPN level determined in our study, it is felt the lower level will provide greater reliability for commercial operations.

2. Prior to arrival of the clams at the plant, the U.V. system and depuration tanks should be thoroughly hosed down with fresh water and the U.V. bulbs cleaned and checked.

3. The U.V. system and pump should be turned on as soon as the clams arrive at the plant. This will allow time, during the culling of the clams, for the system to flush itself out and ensure clean U.V. treated seawater for the depuration process.

4. The clams should be thoroughly washed and culled on a mechanical clam-washer to ensure that only clean, undamaged clams enter the depuration process.

5. Loading of the depuration baskets should be carefully monitored by the plant operator to ensure against over-filling. For this operation, the baskets were designed to hold 0.5 bushel of shellstock when filled to 70% of capacity.

6. A flow of 0.5 gpm/bu should be maintained for each depuration tank.

7. All depuration runs should consist of two cycles; one of 18 and one of 24 hours duration. After both cycles the tanks, baskets, spacers and clams should be thoroughly hosed down.

8. 0 Hour for each depuration run should coincide, as closely as possible, to the time of high tide.

9. When depuration is taking place, the U.V. treated seawater should be sampled daily for temperature, salinity, turbidity, dissolved oxygen, pH, and faecal coliform level prior to entering the depuration tank(s).

10. Constancy of operations should be monitored by means of recording flow-meters and a clock system; the latter consisting of two electric clocks, one of which remains off after any break in electrical service.

Equipment Design and Function

1. If additional sterilization capacity is required from the U.V. equipment, it can be modified to bring it closer to standard design criteria.

2. In a multi-tank system it would be easier to distribute the flow evenly to each flow-meter box if separate pipes ran from the sterilizer to each box rather than the present system wherein a single pipe feeds a series of three boxes.

3. To eliminate flooding of the depuration room floor when tanks are drained, a pipe should connect the tank drain to the in-floor drainage trough. In addition, the drainage trough should be enlarged to accommodate simultaneous draining of all tanks.

4. Tank design could be modified to improve the water flow distribution by eliminating short-circuits or dead spots. This would reduce depuration variability and increase the reliability of sampling results.

5. To assist removal of debris during the depuration process, the tanks could be modified to provide sloped drainage from the sides to the centre of the tank.

6. Although the depuration baskets used in this study proved generally satisfactory, it would be better, in a fully commercial operation, to use baskets built of sturdier material; baskets

which hold their shape better when loaded and which are capable of withstanding heavy usage over an extended period of time.

7. Tank loading and unloading operations could be made more efficient through palletization of the baskets and the use of overhead cranes.

General

1. The Elevated Temperature Coliform Plate Count technique should be verified for use in the Digby plant in order to provide a more rapid means of determining the success of depuration for each lot processed.

2. More reliable water quality guidelines, ideally species specific, should be developed for areas being considered for depuration. This would allow more accurate definition of those areas likely to be suitable for harvesting of stocks for depuration.

3. Further work should be done in mapping the faecal coliform levels for soft-shell clam stocks from the various closed areas of the Basin.

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TABLE 1. Monitoring of physical and chemical factors of Ultra-Violet treated seawater.

Trial No.	Date			Tide	Temperature	Salinity	Dissolved Oxygen
	Day	Month	Year		C	‰	mg/l
1	18	06	75	5h to Low	8.2	33.7	10
1	19	06	75	Low	8.7	33.7	10
1	20	06	75	5h to Low	10.5	32.0	10
2	23	06	75	5h to Low	8.7	27.2	10
2	24	06	75	3h to High	9.2	33.2	10
2	25	06	75	4½h to High	9.4	33.4	10
2	26	06	75	5h to High	9.3	33.2	10
3	02	07	75	3h to High	no data	33.4	10
3	03	07	75	Low	10.3	31.4	9
3	04	07	75	4h to Low	10.3	24.6	9
3	04	07	75	Low	11.0	24.6	9
4	09	07	75	High	9.0	34.2	10
4	09	07	75	2h to Low	10.0	26.8	10
4	10	07	75	Low	10.0	33.4	9
4	10	07	75	3h to Low	9.7	33.4	10
4	11	07	75	Low	10.0	33.0	10
5	15	07	75	1½h to High	10.5	33.2	10
5	16	07	75	5½h to High	11.1	33.7	9
5	17	07	75	2h to Low	10.8	33.4	10
6	30	07	75	3h to High	11.9	34.0	9
6	31	07	75	1h to Low	12.2	32.9	10
6	01	08	75	2h to Low	12.9	32.7	10
7	06	08	75	3h to Low	11.7	32.4	9
7	07	08	75	2½h to Low	11.6	33.2	8
7	08	08	75	2½h to Low	11.6	33.4	9
8	13	08	75	½h to Low	11.1	32.4	10
8	14	08	75	2h to Low	11.1	32.4	10
8	15	08	75	2h to Low	11.4	32.4	9
9	20	08	75	High	11.3	33.4	9
9	21	08	75	Low	11.9	33.2	9
9	22	08	75	3h to High	12.0	33.4	9

TABLE 2. Monitoring of the bacteriological quality of the seawater. Results are expressed in MPN Faecal Coliform per 100 ml of water. A minimum of 3 analyses was performed on each type of water. For U.V. treated and effluent water, the number in parentheses indicates the number of times that result was obtained.

Trial	Date			Tide	Seawater		
No.	Day	Month	Year		Raw	Treated	Effluent
1	18	06	75	5h to Low	6, 13, 49	49, 7, 22[1]	[8]<2, 2
1	19	06	75	Low	8, 29, 47	[3]<2	[9]<2
1	20	06	75	5h to Low	<2, 2, 2	[3]<2	[9]<2
2	23	06	75	5h to Low	<2, <2, 8	[3]<2	[3]<2
2	24	06	75	3h to High	11, 8, 17	[3]<2	2, 5, <2
2	25	06	75	4½h to High	7, 5, 4	[3]<2	[3]<2
2	26	06	75	5h to High	2, 2, 5	[3]<2	[2]<2, 2
3	02	07	75	3h to High	<2, <2, <2	[3]<2	no data
3	03	07	75	Low	4, 2, 9	[3]<2	[9]<2
3	04	07	75	4h to Low	11, 13, 33	[3]<2	[9]<2
3	04	07	75	Low	no data	[3]<2	no data
4	09	07	75	High	<2, 2, 7	[3]<2	no data
4	09	07	75	2h to Low	no data	[3]<2	no data
4	10	07	75	Low	70, 24, 49	[3]<2	[8]<2, 2
4	10	07	75	3h to Low	no data	[3]<2	no data
4	11	07	75	Low	33, 49, 48	[3]<2	[9]<2
5	15	07	75	1½h to High	<2, 2, 2	[3]<2	no data
5	16	07	75	5½h to High	7, 8,130	[3]<2	[3]<2, [2]2, 5
5	17	07	75	2h to Low	no data	[3]<2	[3]<2
	22	07	75	4h to Low	2, 5, 8	[3]<2	no data
6	30	07	75	3h to High	<2, <2, <2	[3]<2	[11]<2, 2
6	31	07	75	1h to Low	130, 79, 79	[3]<2	[3]<2
6	01	08	75	2h to Low	5, 8, 23	[3]<2	[2]<2, 5
7	06	08	75	3h to Low	<2, <2, 4	[3]<2	[5]<2, 7 [2]2, 8
7	07	08	75	2½h to Low	17, 11, 4	[3]<2	[9]<2
7	08	08	75	2½h to Low	33, 70, 21	[3]<2	[9]<2
8	13	08	75	½h to Low	2, 9, 7	[3]<2	[9]<2
8	14	08	75	2h to Low	14, 17, 8	[3]<2	[6]<2, [2]2, 5
8	15	08	75	2h to Low	13, 27, 22	[3]<2	[9]<2
9	20	08	75	High	<2, <2, <2	[3]<2	[8]<2, 5
9	21	08	75	Low	12, 21, 7	[3]<2	[9]<2
9	22	08	75	3h to High	5, 4, 6	[3]<2	[9]<2

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[1] The U.V. system was not operating at the time this sample was taken.

TABLE 3. Trial 1. 18-20 June. Min, Md, Max stand for Minimum, Median, Maximum respectively.

			MPN Faecal Coliform/100g									
Tank	Loading	Flow-rate	0 Hour			18 Hour			42 Hour			
No.	(no. baskets)	(gpm/bu)	Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	42	0.5	130	170	490	50	170	1700	<20	<20	130	Tank drained & hosed down Baskets not hosed down
2	42	0.7	170	595	790	50	100	220	<20	20	330	Tank drained & hosed down Baskets not hosed down
3	42	1.0	120	700	790	80	130	130	40	840	2200	Tank drained & hosed down Baskets not hosed down

TABLE 4. Trial 2. 23-26 June.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g												
			0 Hour			24 Hour			48 Hour			72 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	42	0.5	330	490	790	<20	20	140	<20	70	5400	<20	35	700	Tank drained & hosed down Baskets not hosed down

TABLE 5. Trial 3. 02-04 July.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g									
			0 Hour			18 Hour			42 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	42	1.0	80	105	170	<20	20	80	20	150	490	Tank drained & not hosed down Baskets not hosed down
2	42	1.0	170	700	2200	<20	30	80	20	30	80	Tank drained & hosed down Baskets hosed down
3	42	0.5	170	325	790	<20	<20	20	<20	35	80	Tank drained & hosed down Baskets hosed down

TABLE 6. Trial 4. 09-11 July.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g									
			0 Hour			18 Hour			42 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	42	0.5	2400	20000	24000	50	140	790	1300	7300	24000	Tank drained & hosed down Baskets hosed down
2	42	0.5	220	4450	24000	50	130	170	1100	3550	16000	Tank drained & hosed down Baskets hosed down
3	42	1.0	790	2250	9200	130	2800	5400	230	5400	16000	Tank drained & hosed down Baskets hosed down

TABLE 7. Trial 5. 15-17 July.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g									
			0 Hour			18 Hour			42 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	36	0.5	16000	20000	24000+	120	230	2100	16000	16000	24000+	Tank drained & hosed down Baskets hosed down held dry 4 hours
2	36	0.5	2200	3900	16000	330	595	3500	1300	2400	5400	Tank drained & hosed down Baskets hosed down held dry 4 hours
3	42	0.5	130	4830	16000	130	280	1300	330	640	16000	Tank drained & hosed down Baskets hosed down held dry 4 hours

TABLE 8. Trial 6. 30 July-01 August.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g									
			0 Hour			18 Hour			42 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	
3	30	0.5	330	640	1300	790	1045	3500	790	3500	24000+	Tank not drained & not hosed down Baskets not hosed down

TABLE 9. Trial 7. 06-08 August.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g									
			0 Hour			18 Hour			42 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	
1	42	0.5	330	330	490	120	400	2200	140	390	16000	No disinfection Tank drained & hosed down Baskets hosed down
2	28	0.5	330	330	330	<20	60	110	<20	20	130	No disinfection Tank drained & hosed down Baskets hosed down
3	28	0.5	220	270	1100	<20	65	490	50	130	230	Disinfection Tank drained & hosed down Baskets hosed down

TABLE 10. Trial 8. 13-15 August.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g											
			0 Hour			18 Hour			30 Hour			42 Hour		
			Min	Md	Max	Min	Md	Max	Min	Md	Max	Min	Md	Max
1	28	0.5	130	310	3500	170	410	2400				<20	125	1700
2	28	0.5	790	1100	1300	20	105	330	330	870	3500	<20	35	80
3	28	0.5	490	490	1100	80	200	490				<20	<20	80

1 Tank drained & hosed down. Baskets hosed down.

2 Tank drained & hosed down at 18 and 30 Hour. Baskets hosed down at 18 and 30 Hour.

3 Tank drained & hosed down. Baskets emptied and hosed down. Clams spread out, hosed down and put back in baskets.

TABLE 11. Trial 9. 20-22 August.

Tank no.	Loading (no. baskets)	Flow-rate (gpm/bu)	MPN Faecal Coliform/100g												
			0 Hour			18 Hour			42 Hour						
			Min	Md	Max	Min	Md	Max	Min	Md	Max				
1	28	0.5	310	460	490	<20	90	230	20	150	790				Tank drained & hosed down Baskets hosed down
3	28	0.5	330	460	1300	<20	30	50	<20	35	490				Tank drained & hosed down Baskets emptied & hosed down Clams spread out, hosed down & put back in baskets
			0 Hour			18 Hour			28 Hour			30 Hour			
			Min	Md	Max	Min	Md	Max	Min	Md	Max	Min	Md	Max	
2	28	0.5	1600	5400	24000+	<20	<20	20	<20	<20	20	20	40	80	Tank drained & hosed down at 18 and 30 Hour Baskets hosed down at 18 and 30 Hour
			32 Hour			40 Hour			42 Hour						
			Min	Md	Max	Min	Md	Max	Min	Md	Max				
			20	40	310	330	950	1700	20	140	2400				

TABLE 12. Experimental lots with an end product meeting the criteria for depurated shellfish. Particular procedures pertaining to each lot are listed. Md, Max stand for median, maximum.

Trial - Tank	MPN F.C./100g						Layers baskets	Flow gpm/bu	Load cu.ft/bu	Tank drainage	Tank hosing	Basket hosing
	0 Hour		18-24 Hour		42-48 Hour							
	Md	Max	Md	Max	Md	Max						
1 - 1	170	490	170	1700	<20	130	3	0.5	3.7	+	+	-
1 - 2	595	790	100	220	20	330	3	0.7	3.7	+	+	-
3 - 2	700	2200	30	80	30	80	3	1.0	3.7	+	+	+
3 - 3	325	790	<20	20	35	80	3	0.5	3.7	+	+	+
7 - 2	330	330	60	110	20	130	2	0.5	6.0	+	+	+
8 - 2	1100	1300	105	330	35	80	2	0.5	5.7	+	+	+
8 - 3	490	1100	200	490	<20	80	2	0.5	5.8	+	+	+
9 - 3	460	1300	30	50	35	490	2	0.5	5.7	+	+	+

TABLE 13. Statistical analysis of faecal coliform counts of experimental lots with an end product meeting the criteria for depurated shellfish.

	0 Hour	18-24 Hour	42-48 Hour
N	33	45	45
Range MPN F.C./100g	130 to 2220	<20 to 1700	<20 to 490
Median	490	50	20
Geometric mean	490	53	25
Standard deviation$_{\text{log}}$	0.32	0.54	0.56

TABLE 14. Experimental lots with an end product not meeting the criteria for depurated shellfish and with a low initial bacterial load. Particular operational procedures pertaining to each lot are listed.

Trial - Tank	MPN F.C./100g						Layers baskets	Flow gpm/bu	Load cu.ft/bu	Tank drainage	Tank hosing	Basket hosing
	0 Hour		18-24 Hour		42-48 Hour							
	Md	Max	Md	Max	Md	Max						
1 - 3	700	790	130	130	840	2200	3	1.0	3.7	+	+	-
2 - 1	490	790	20	140	35	700*	3	0.5	3.4	+	+	-
3 - 1	105	170	20	80	150	490	3	1.0	3.7	+	-	-
6 - 3	640	1300	1045	3500	3500	24000+	3	0.5	5.3	-	-	-
7 - 1	330	490	400	2200	390	16000	3	0.5	4.3	+	+	+
7 - 3	270	1100	65	490	130	230	2	0.5	7.1	+	+	+
9 - 1	460	490	90	230	150	790	2	0.5	5.3	+	+	+

* For this particular trial (2 - 1) final counts shown in column of the 42-48 Hour counts correspond to the 72 Hour counts.

TABLE 15. Statistical analysis of faecal coliform counts of experimental lots with an end product not meeting the criteria for depurated shellfish and with a low initial bacterial load.

	0 Hour	18-24 Hour	42-48 Hour
N	30	35	39
Range MPN F.C./100g	80 to 1300	<20 to 3500	<20 to 24000+
Median	460	120	220
Geometric mean	370	110	220
Standard deviation$_{\text{log}}$	0.37	0.82	0.84

TABLE 16. Experimental lots with an end product not meeting the criteria for depurated shellfish and with a high initial bacterial load. Particular operational procedures pertaining to each lot are listed.

Trial - Tank	MPN F.C./100g						Layers baskets	Flow gpm/bu	Load cu.ft/bu	Tank drainage	Tank hosing	Basket hosing
	0 Hour		18-24 Hour		42-48 Hour							
	Md	Max	Md	Max	Md	Max						
4 - 1	20000	24000	140	790	7300	24000	3	0.5	3.7	+	+	+
4 - 2	4450	24000	130	170	3550	16000	3	0.5	3.9	+	+	+
4 - 3	2250	9200	2800	5400	5400	16000	3	1.0	3.9	+	+	+
5 - 1	20000	24000+	230	2100	16000	24000+	3	0.5	4.4	+	+	+
5 - 2	3900	16000	595	3500	2400	5400	3	0.5	4.5	+	+	+
5 - 3	4830	16000	280	1300	640	16000	3	0.5	4.0	+	+	+
8 - 1	310	3500	410	2400	125	1700	2	0.5	5.1	+	+	+
9 - 2	5400	24000+	<20	20	140	2400	2	0.5	6.0	+	+	+

TABLE 17. Biochemical identification of faecal coliform isolates collected during trial 5. The analysis was carried out by the Environmental Protection Service.

TRIAL NUMBER: 5

DATE: 15-17 JULY, 1975

Period of Depuration (hours)	*Escherichia coli*	Klebsiella Types	Enterobacter Types	Citrobacter Types	No. of Isolates
0	7 (35 %)	5 (25 %)	6 (30 %)	2 (10 %)	20
18	11 (39.3%)	11 (39.3%)	5 (17.8%)	1 (3.6%)	28
42	8 (30.7%)	15 (57.5%)	2 (7.7%)	1 (3.8%)	26
TOTAL	26 (35.1%)	31 (41.9%)	13 (17.6%)	4 (5.4%)	74

TABLE 18. Biochemical identification of faecal coliform isolates collected during trial 6. The analysis was carried out by the Environmental Protection Service.

TRIAL NUMBER: 6

DATE: 30 JULY - 1 AUGUST, 1975.

Period of Depuration (hours)	*Escherichia coli*	Klebsiella Types	Enterobacter Types	Citrobacter Types	No. of Isolates
Shellstock sample prior to depuration	11 (57.9%)	3 (15.8%)	5 (26.3%)	0 (0 %)	19
0	14 (45.2%)	8 (25.8%)	6 (19.4%)	3 (9.7%)	31
18	10 (45.4%)	10 (45.5%)	2 (9.1%)	0 (0 %)	22
42	3 (12 %)	15 (60 %)	6 (24 %)	1 (4 %)	25
TOTAL	38 (39.2%)	36 (37.1%)	19 (19.6%)	4 (4.1%)	97

TABLE 19. Faecal coliform MPN's and biochemical identification of faecal coliform isolates collected by swab sampling during trial 8. The analysis was carried out by the Environmental Protection Service. Note that the value <20MPN is the degree of sensitivity of the test used.

Location of sample	Depuration time (hours)			
	18		42	
	Before hosing down	After hosing down	Before hosing down	After hosing down
	MPN F.C./100ml		MPN F.C./100ml	
Shell surface of clam	20	<20	<20	<20
	80	<20	<20	<20
Bottom of wire mesh basket	20	<20	110	<20
	130	<20	50	<20
Bottom of tank	120	<20	20	<20
	50	<20	20	<20

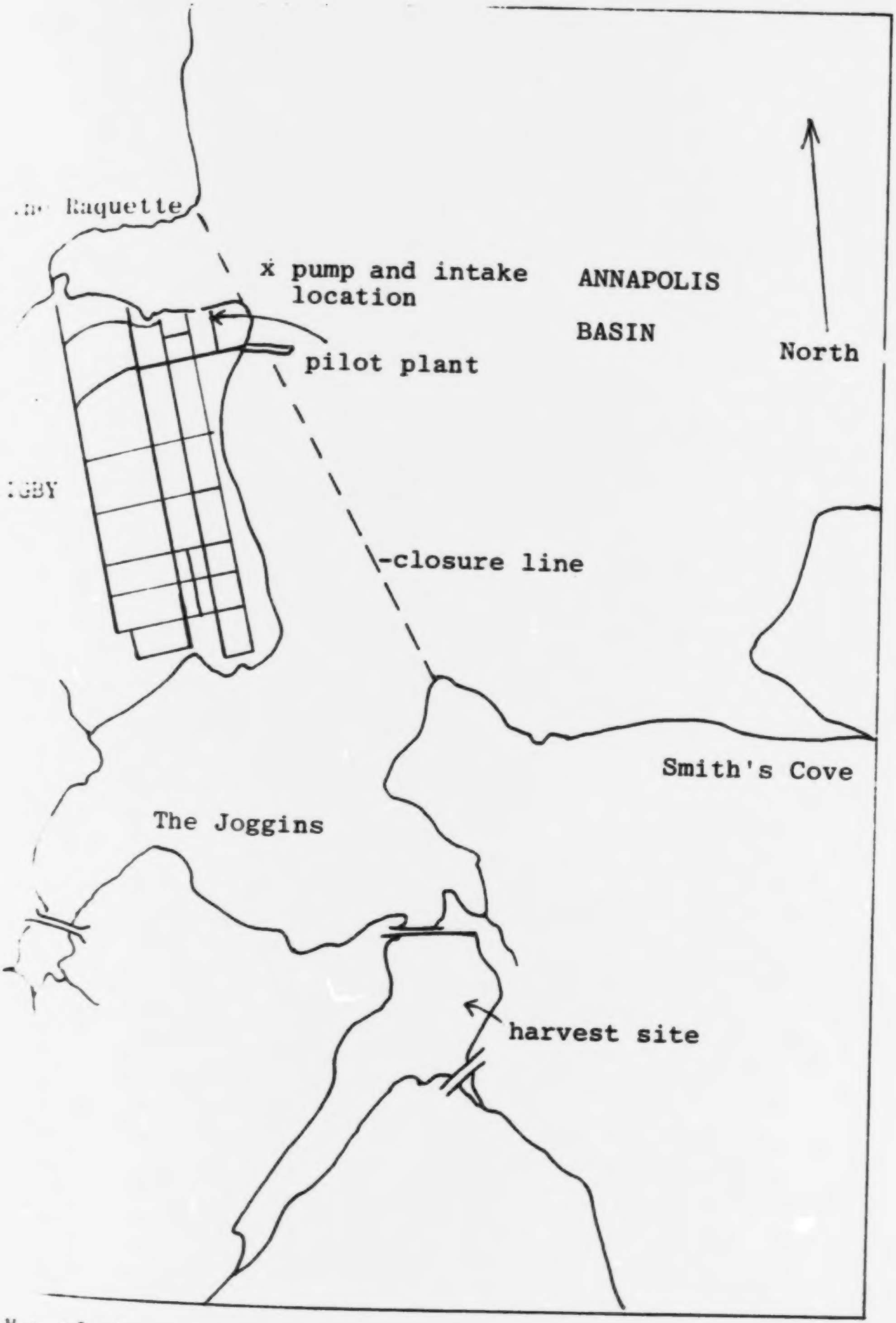
Location of sample	Period of depuration (hours)	*Escherichia Coli*	Klebsiella types	Enterobacter types	No. of isolates
Shell surface of clam	18	2	1	1	4
Bottom of wire mesh basket	18	3	0	1	4
	42	2	3	1	6
Bottom of tank	18	2	1	2	5
	42	1	0	1	2
	TOTAL	10 (48%)	5 (24%)	6 (28%)	21

TABLE 20. Bacteriological counts of shellstock samples collected on certain dates during the summer.

Trial No.	Date			MPN F.C./100g
	Day	Month	Year	
[illegible]	15	07	75	9200
				24000+
[illegible]	23	07	75	700
				700
				700
				2200
				2200
				3500
[illegible]	30	07	75	1700
				5400
				9200
				16000
[illegible]	06	08	75	220
				490
				700
				1300
[illegible]	13	08	75	330
				330
				490
				700

TABLE 21. **Median MPN values derived from the shellstock counts, the 0 Hour counts, and the shellstock plus 0 Hour counts.**

	No. of samples		**Median (MPN)**
Shellstock counts (July 15-Aug. 13)			
Below 1000 MPN F.C./100g	10		595
Above 1000 MPN F.C./100g	10		4450
	20		
0 Hour counts (June 18-Aug. 20)			
Below 2000 MPN F.C./100g	73		480
Above 2000 MPN F.C./100g	32		9200
	105		
Shellstock + 0 Hour counts			
Below 2000 MPN F.C./100g	85		490
Above 2000 MPN F.C./100g	40		9200
	125		


Raquette
x pump and intake location
ANNAPOLIS
BASIN
North
pilot plant
IGBY
-closure line
Smith's Cove
The Joggins
harvest site

of the south-west end of the Annapolis Basin showing of the plant, pump and intake location, closure line site.

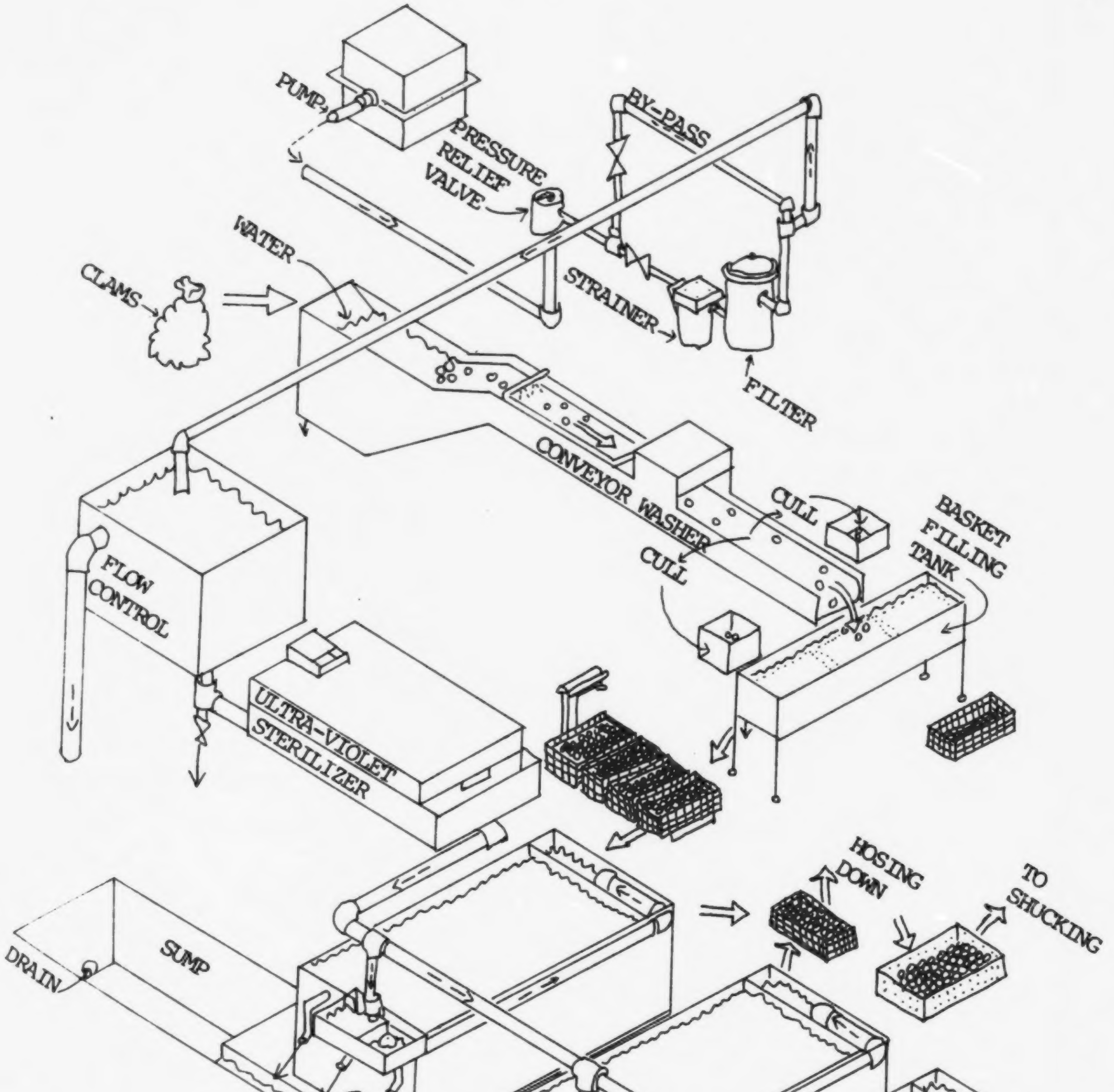
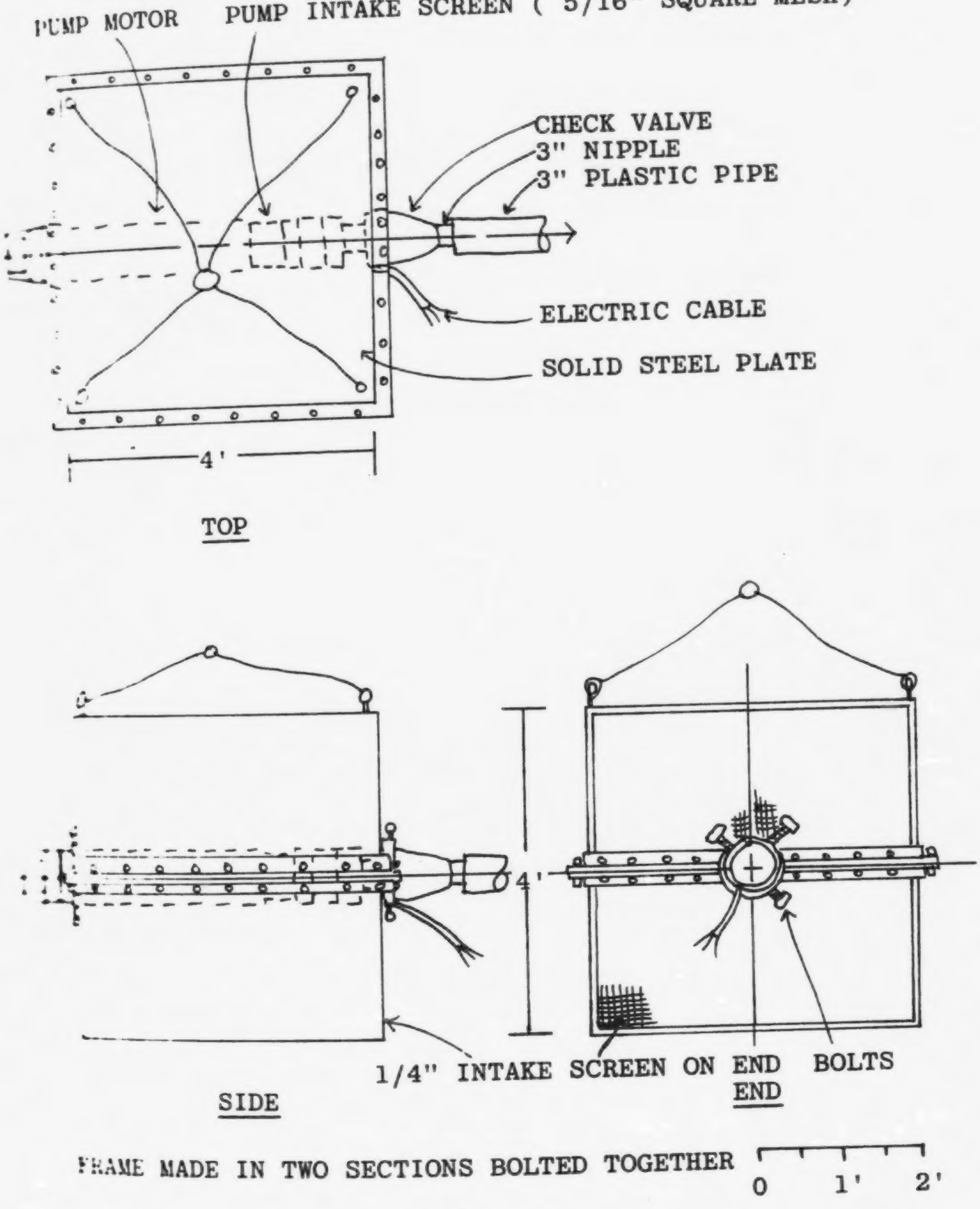

PUMP
PRESSURE RELIEF VALVE
BY-PASS
STRAINER
FILTER
WATER
CLAMS
CONVEYOR WASHER
CULL
CULL
BASKET FILLING TANK
FLOW CONTROL
ULTRA-VIOLET STERILIZER
HOSING DOWN
TO SHUCKING
SUMP
DRAIN
DEPURATION TANK

FIG. 2. Lay-out of the equipment used for seawater treatment and clam processing.


PUMP MOTOR
PUMP INTAKE SCREEN (5/16" SQUARE MESH)
CHECK VALVE
3" NIPPLE
3" PLASTIC PIPE
ELECTRIC CABLE
SOLID STEEL PLATE
4'
TOP
4'
1/4" INTAKE SCREEN ON END
END
BOLTS
SIDE
END
FRAME MADE IN TWO SECTIONS BOLTED TOGETHER
0
1'
2'

Details of the submersible pump used.

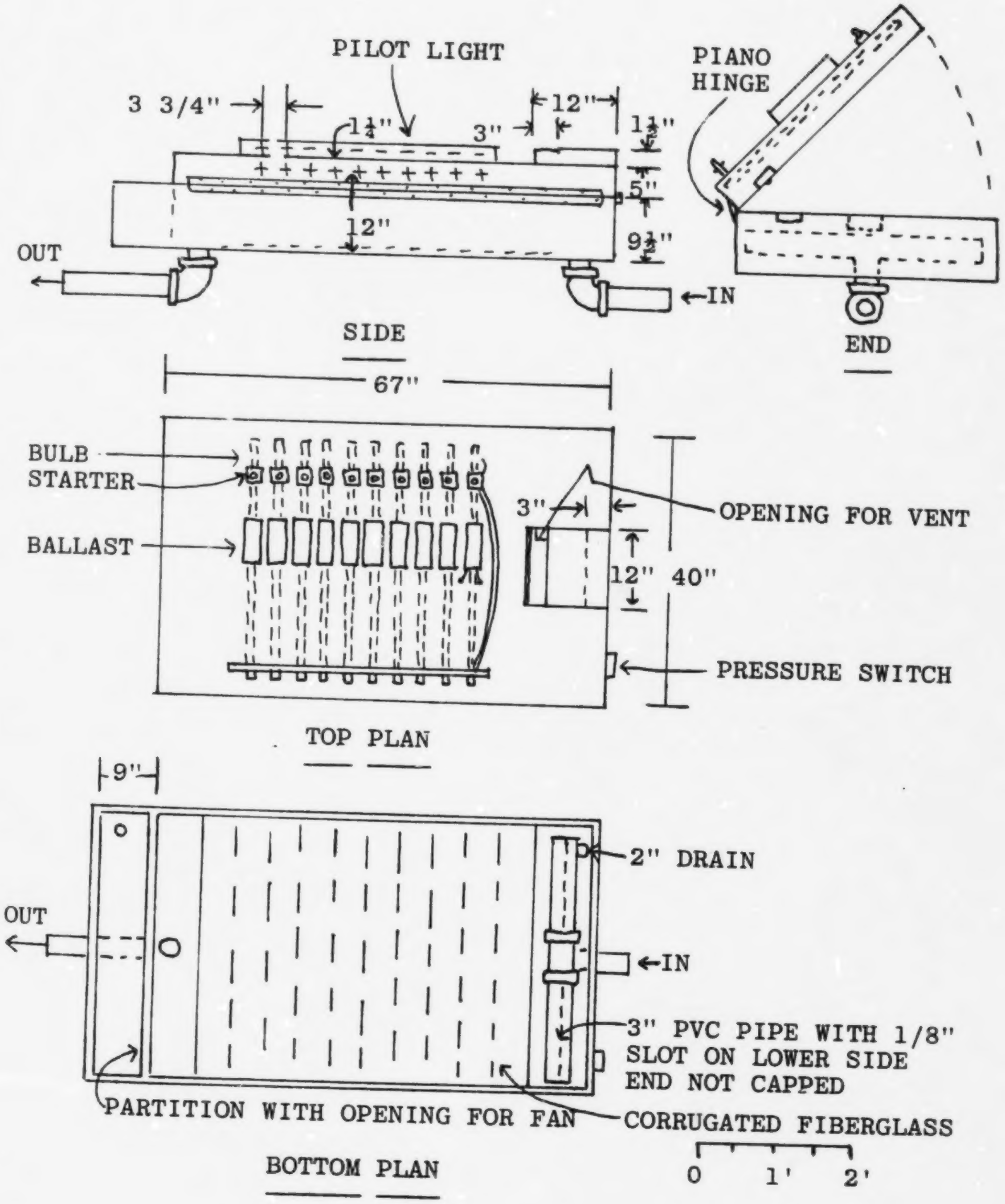

PILOT LIGHT
PIANO HINGE
3 3/4"
1¼"
12"
3"
1½"
5"
12"
9½"
OUT
IN
SIDE
END
67"
BULB
STARTER
BALLAST
3"
OPENING FOR VENT
12"
40"
PRESSURE SWITCH
TOP PLAN
9"
OUT
2" DRAIN
IN
3" PVC PIPE WITH 1/8" SLOT ON LOWER SIDE END NOT CAPPED
PARTITION WITH OPENING FOR FAN
CORRUGATED FIBERGLASS
BOTTOM PLAN
0
1'
2'

FIG. 4. Details of the Ultra-Violet sterilizer.

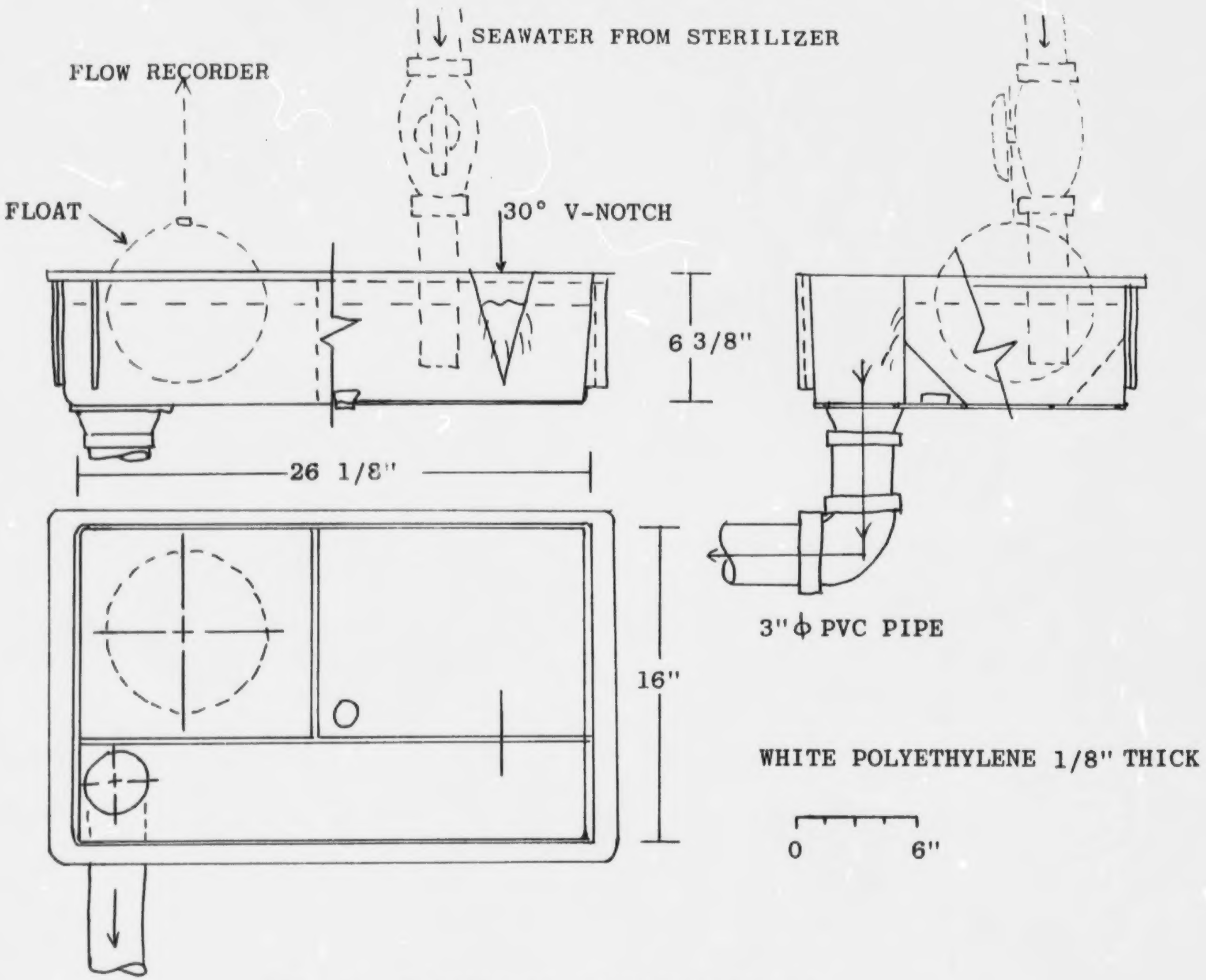
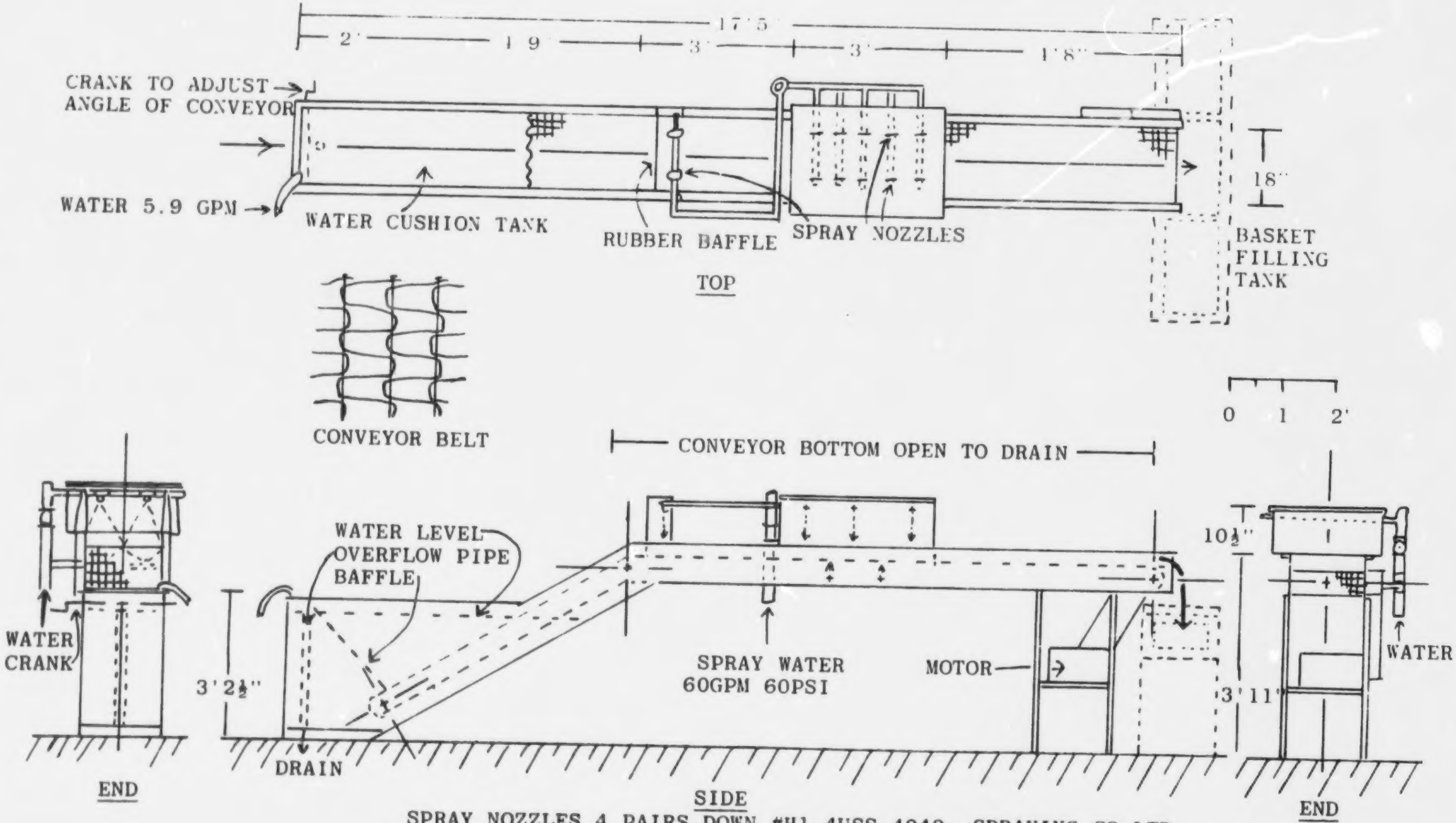
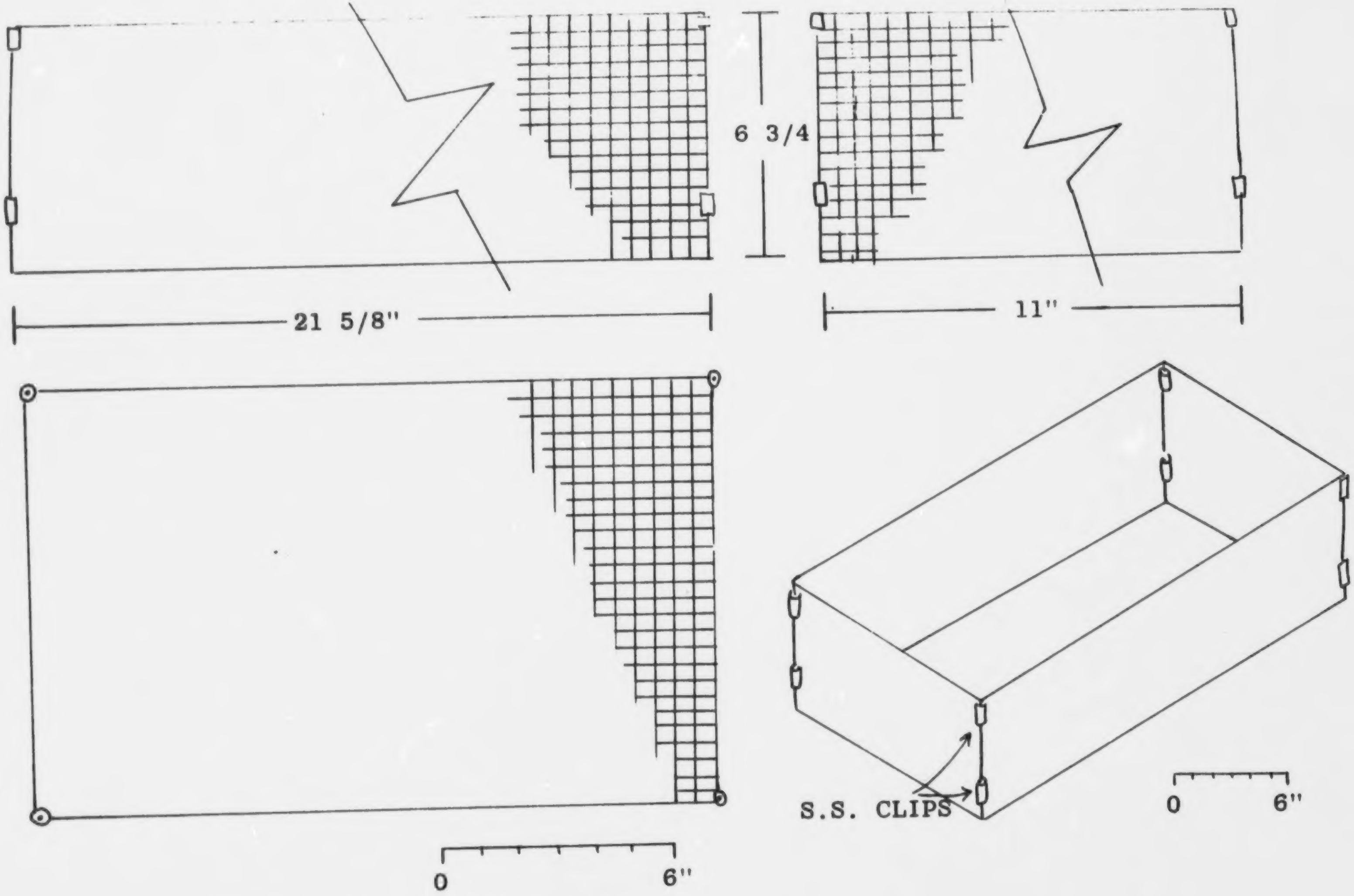

SEAWATER FROM STERILIZER
FLOW RECORDER
FLOAT
30° V-NOTCH
6 3/8"
26 1/8"
16"
3" ϕ PVC PIPE
WHITE POLYETHYLENE 1/8" THICK
0
6"

FIG. 5. Details of a flow-meter box.


17'5"
2'
1'9"
3'
3'
1'8"
CRANK TO ADJUST ANGLE OF CONVEYOR
WATER 5.9 GPM
WATER CUSHION TANK
RUBBER BAFFLE
SPRAY NOZZLES
18"
BASKET FILLING TANK
TOP
CONVEYOR BELT
0 1 2'
CONVEYOR BOTTOM OPEN TO DRAIN
WATER CRANK
END
WATER LEVEL
OVERFLOW PIPE
BAFFLE
3'2½"
DRAIN
SPRAY WATER 60GPM 60PSI
MOTOR
10½"
3'11"
WATER
END
SIDE

SPRAY NOZZLES 4 PAIRS DOWN #H1-4USS-4040 SPRAYING CO LTD
2 " UP #H1-4USS-9540 STRAIGHT LINE PATTERN
CONVEYOR BELT MESH ½"x1" USDA APPROVED 24 FPM 316. STAINLESS STEEL
DESIGNED BY: STERL, DE ROCHER, HURST, 1964, MAINE
BUILT BY: FRANK LARCHE, DIGBY, N.S. 1975. #CW 457.

FIG. 6. Details of the conveyor clam-washer.


6 3/4
21 5/8"
11"
S.S. CLIPS
0
6"
0
6"

DIMENSIONS (INCHES): 11 x 21 5/8 x 6 3/4
SCREEN MESH (INCHES): 1/2 x 1/2
FIG. 7. Details of a depuration basket.

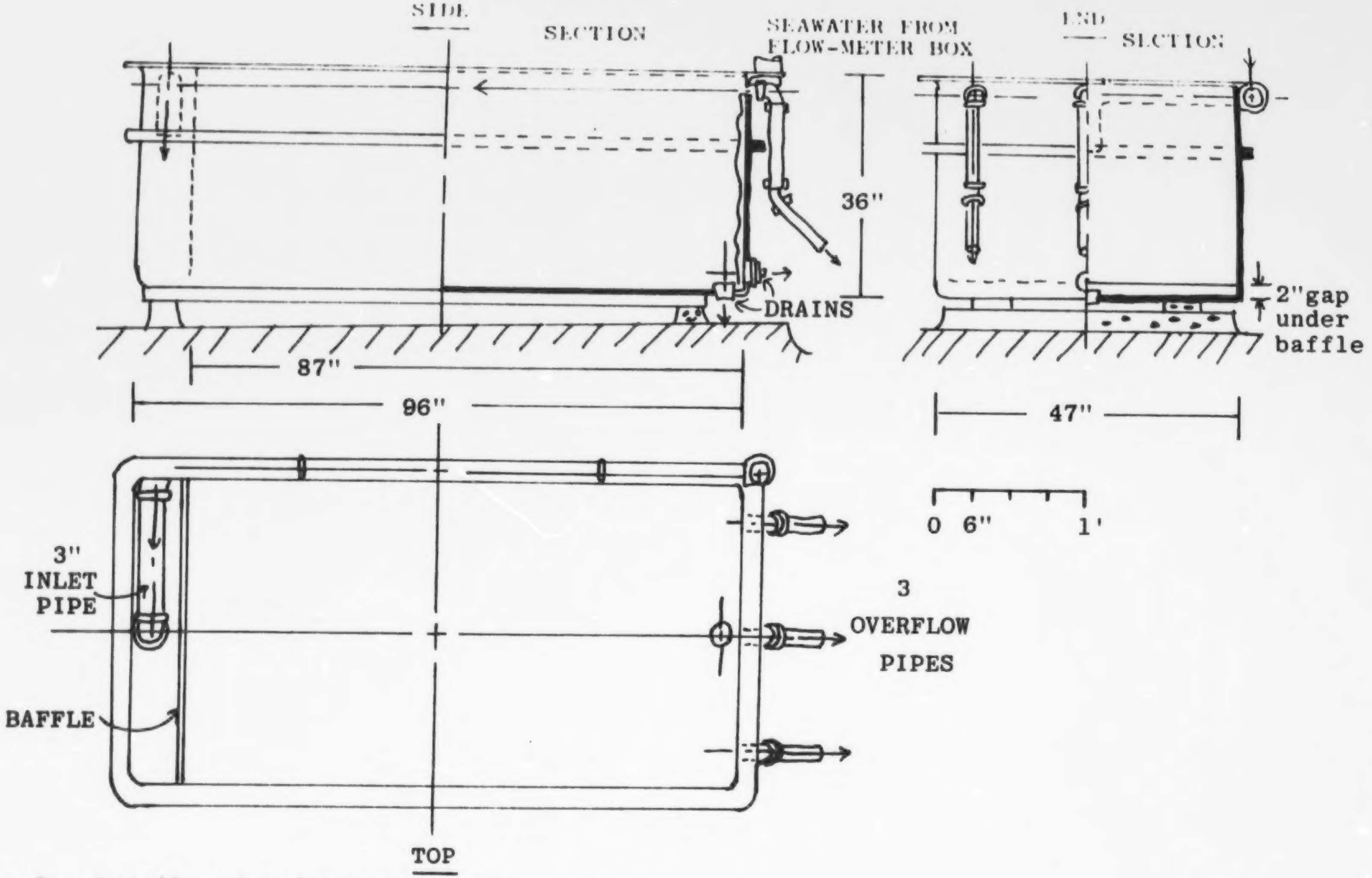

SIDE
SECTION
SEAWATER FROM
FLOW-METER BOX
END
SECTION
36"
DRAINS
2"gap
under
baffle
87"
96"
47"
0 6" 1'
3"
INLET
PIPE
3
OVERFLOW
PIPES
BAFFLE
TOP

FIG. 8. Details of a depuration tank.

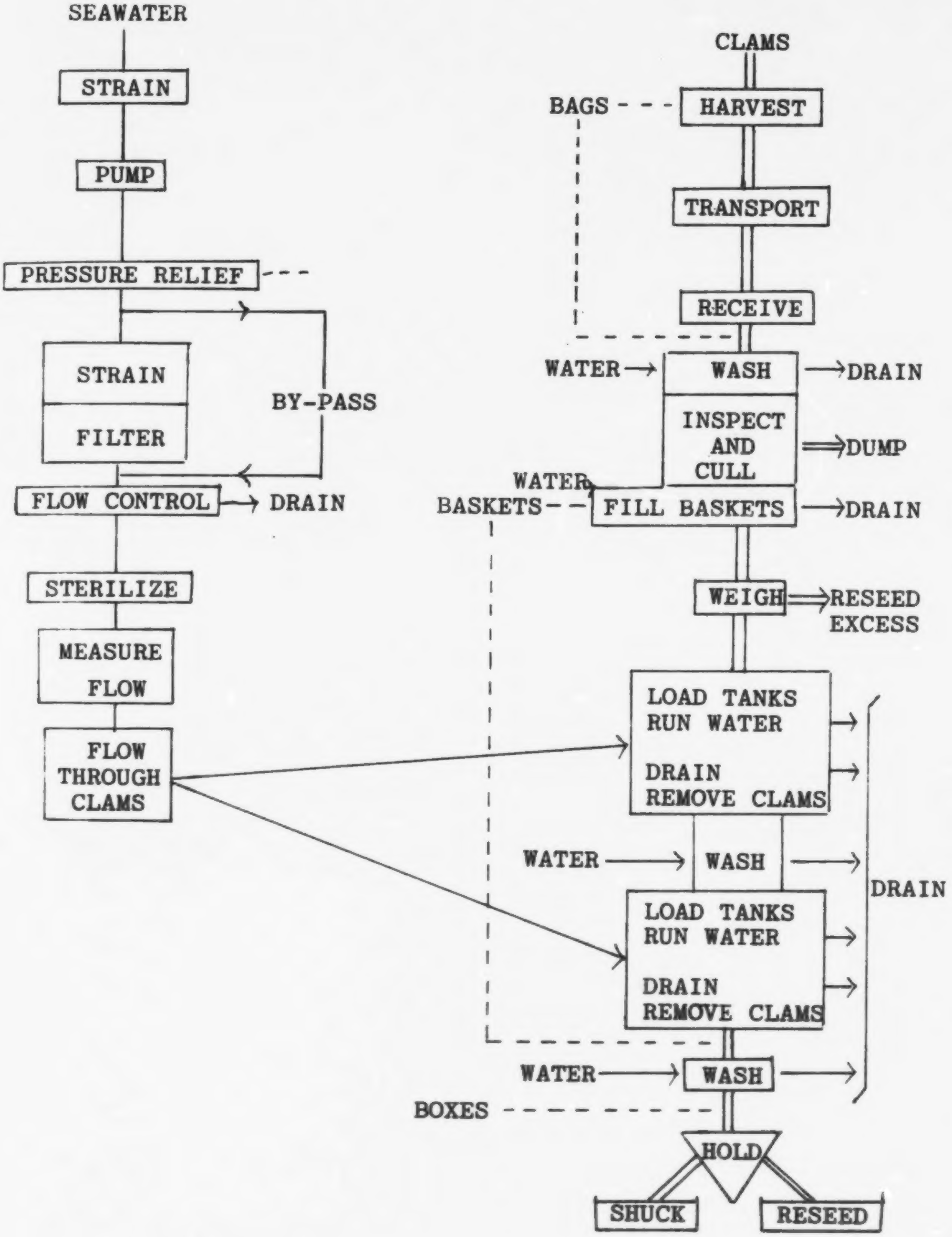

SEAWATER
STRAIN
PUMP
PRESSURE RELIEF
STRAIN
FILTER
BY-PASS
FLOW CONTROL
DRAIN
STERILIZE
MEASURE FLOW
FLOW THROUGH CLAMS
CLAMS
BAGS
HARVEST
TRANSPORT
RECEIVE
WATER
WASH
DRAIN
INSPECT AND CULL
DUMP
WATER
BASKETS
FILL BASKETS
DRAIN
WEIGH
RESEED EXCESS
LOAD TANKS
RUN WATER
DRAIN
REMOVE CLAMS
WATER
WASH
DRAIN
LOAD TANKS
RUN WATER
DRAIN
REMOVE CLAMS
WATER
WASH
BOXES
HOLD
SHUCK
RESEED

FIG. 9 . Flow-chart of the depuration plant operations.

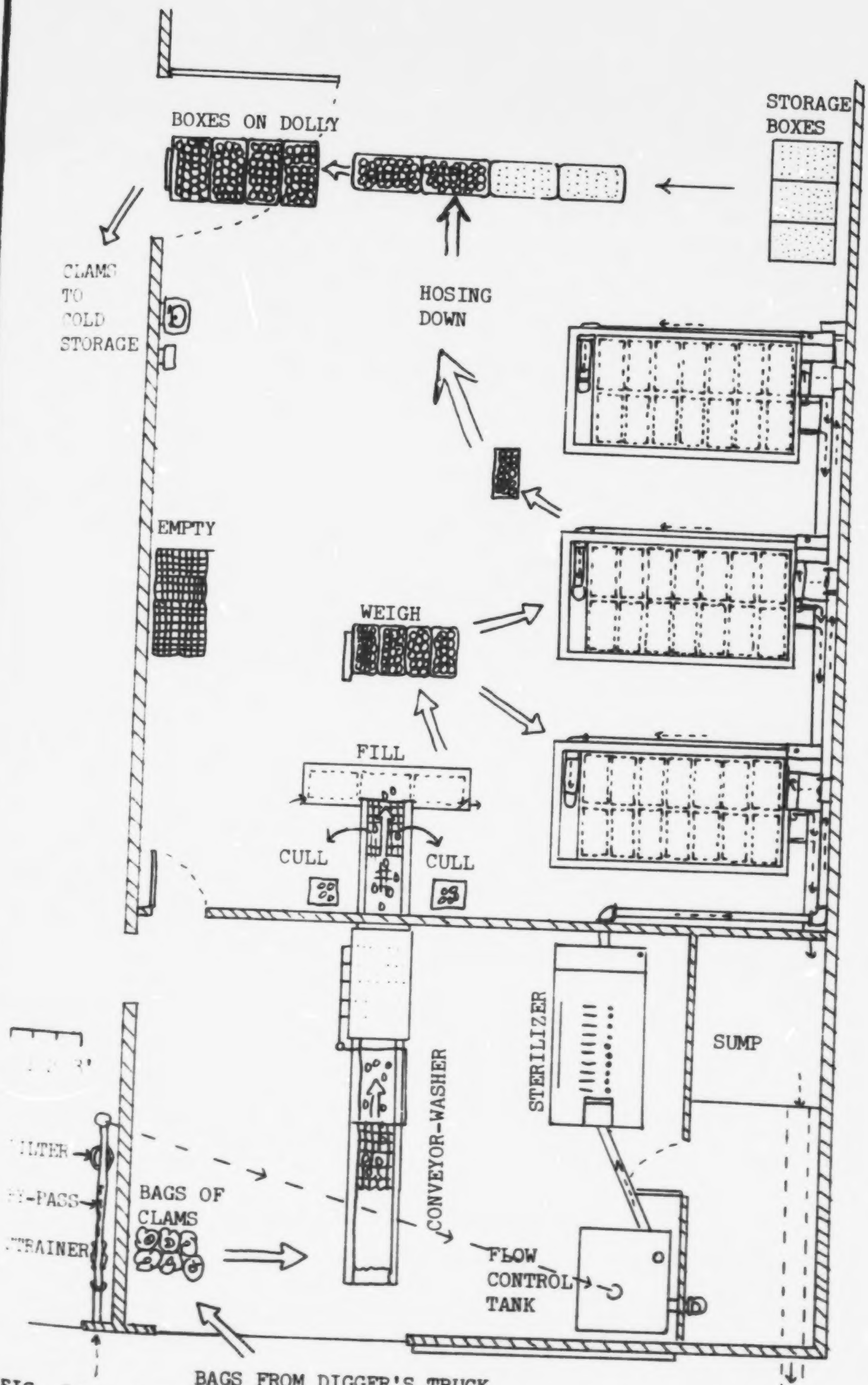

BOXES ON DOLLY
STORAGE BOXES
CLAMS TO COLD STORAGE
HOSING DOWN
EMPTY
WEIGH
FILL
CULL
CULL
STERILIZER
SUMP
CONVEYOR-WASHER
FILTER
BY-PASS
STRAINER
BAGS OF CLAMS
FLOW CONTROL TANK
BAGS FROM DIGGER'S TRUCK

FIG. 10. Lay-out of the equipment. The order in which the different operations take place is also illustrated.

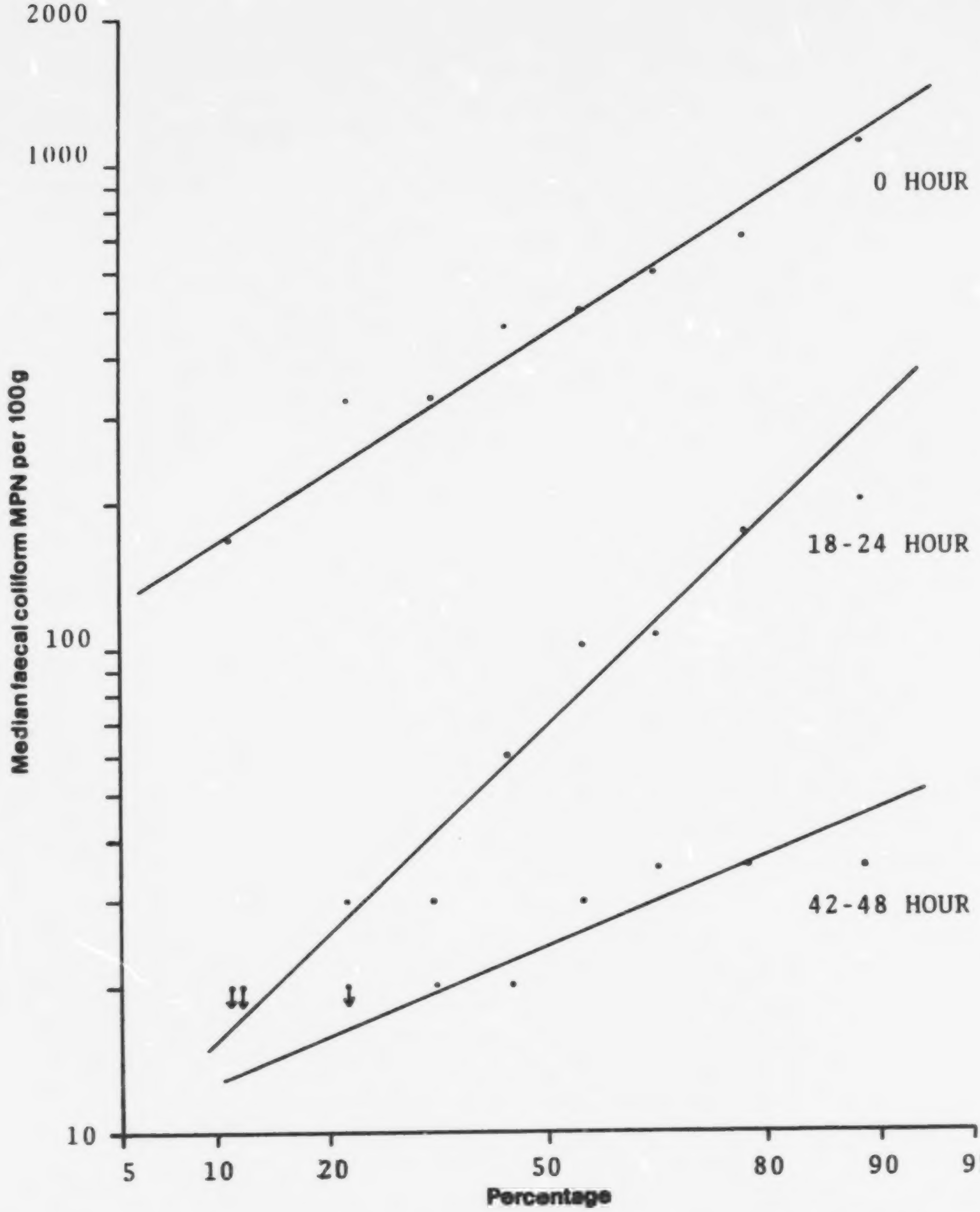


FIG. 11. Probability plots of faecal coliform counts (MPN) at different periods of depuration time for experimental lots with an end product meeting the criteria for depurated shellfish. ↓ indicates "less than".

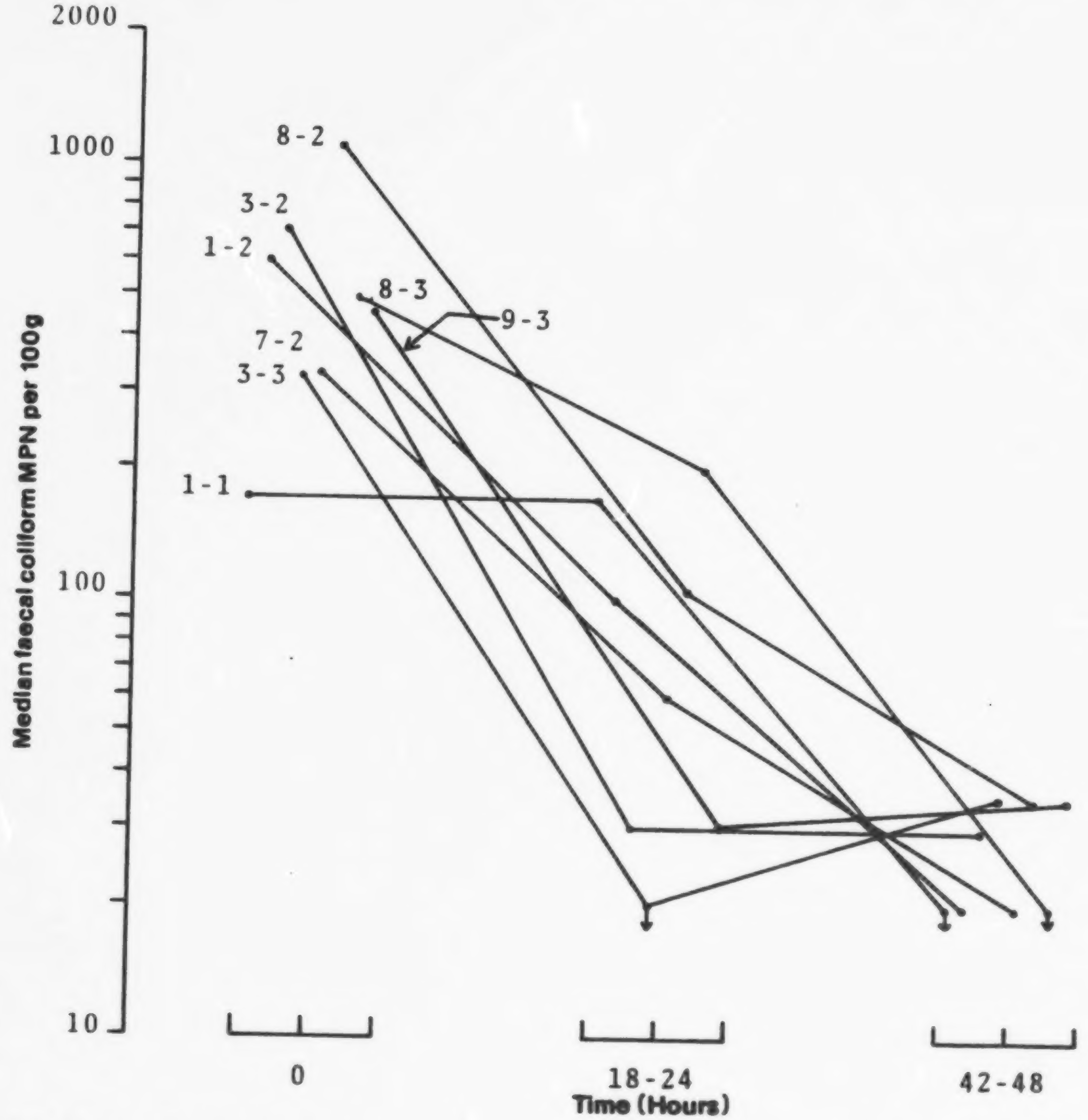


FIG. 12. Bacterial reduction versus time of depuration for the different experimental lots referred to under Group 1.

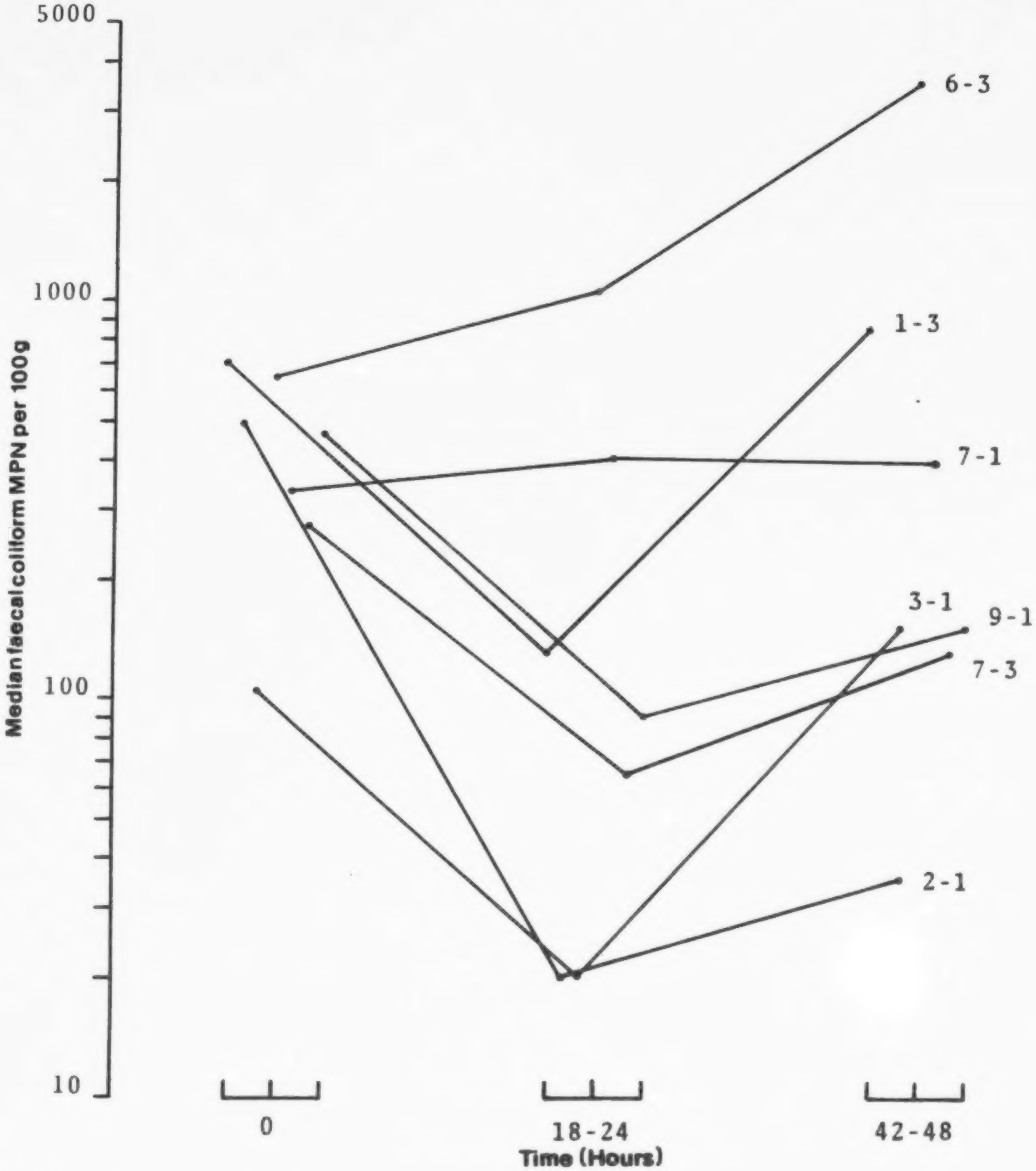


FIG. 13. Bacterial fluctuations versus time of depuration for the different experimental lots referred to under Group 2.

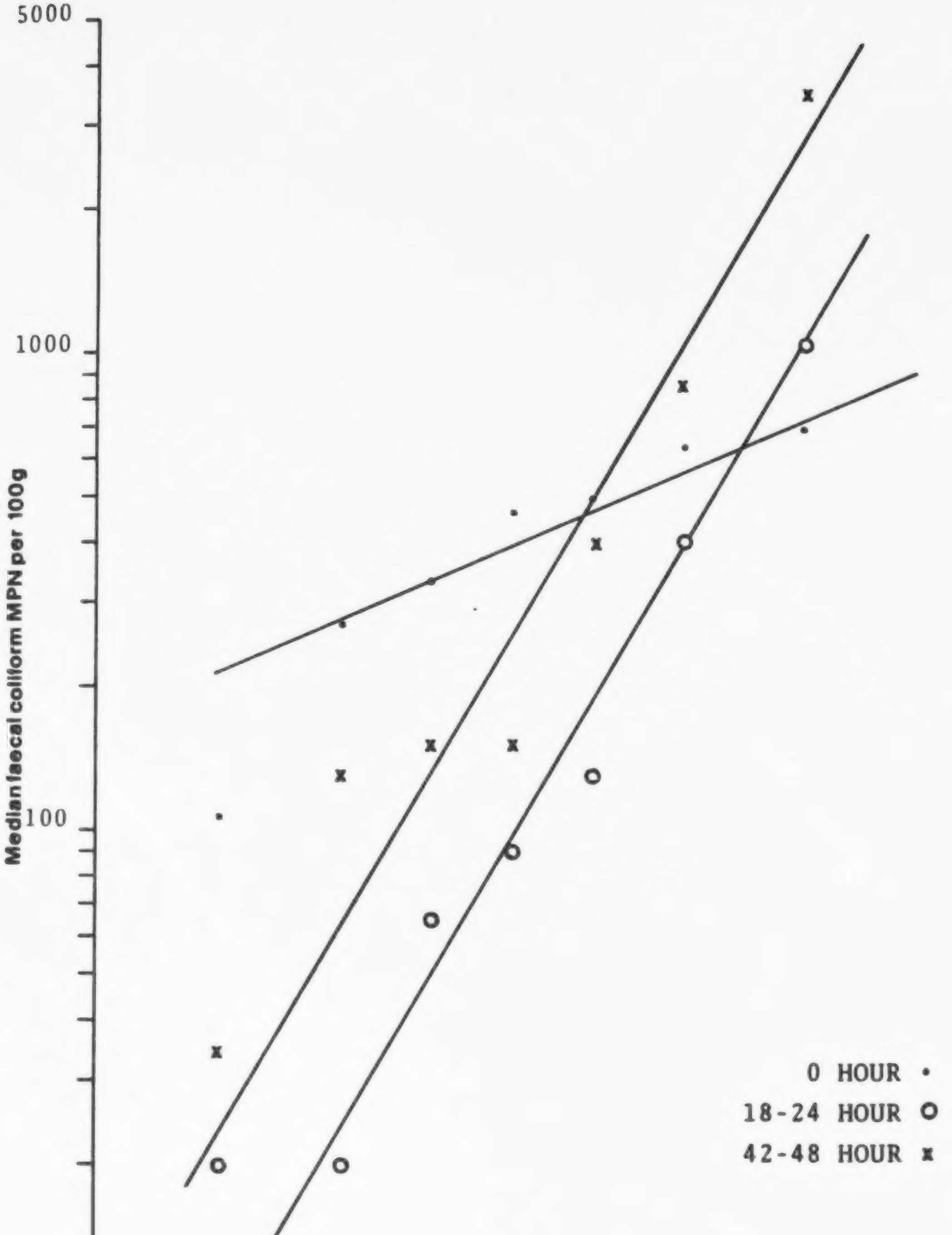


FIG. 14. Probability plots of faecal coliform counts (MPN) at different periods of depuration time for the experimental lots referred to under Group 2.

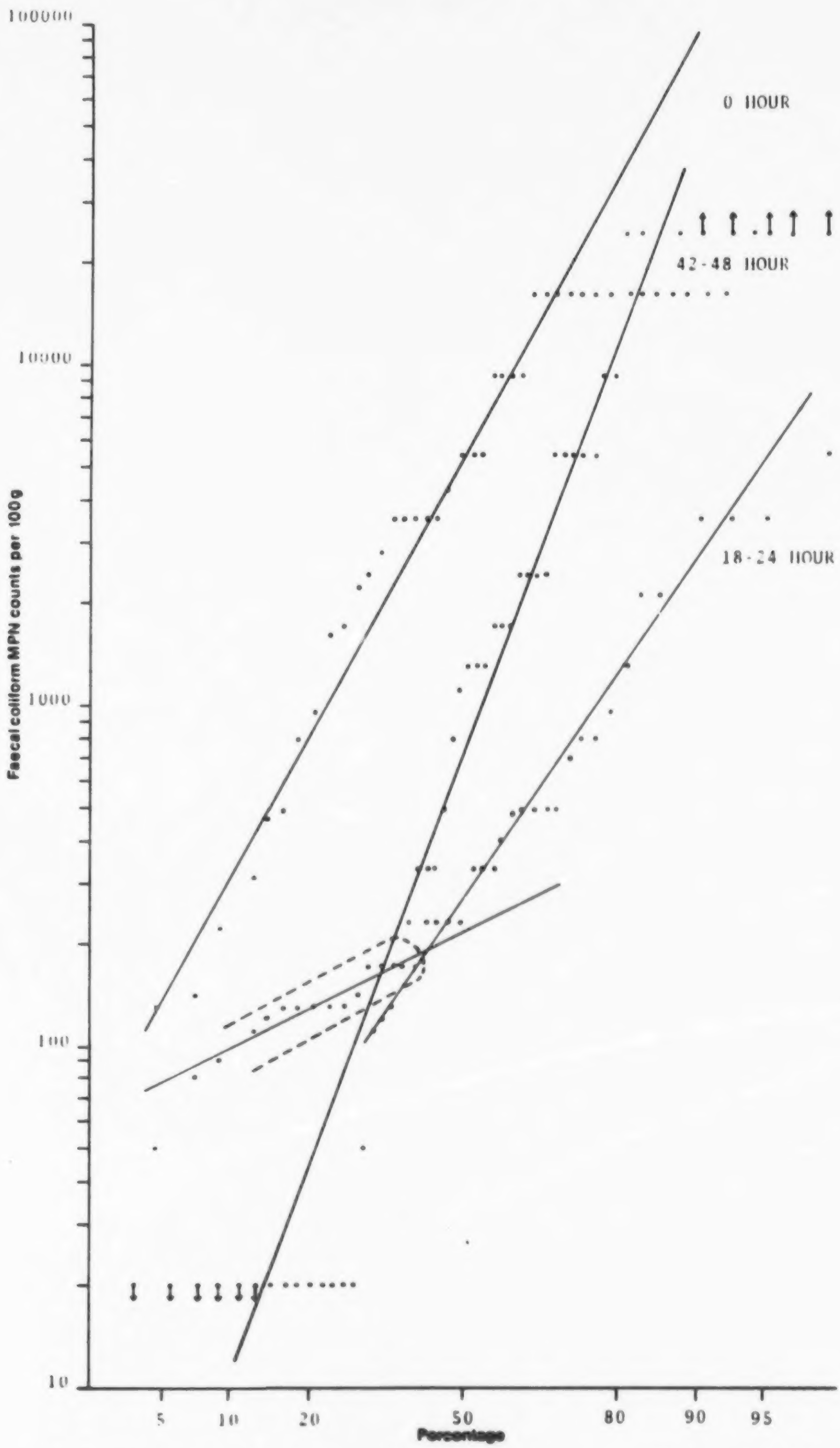


FIG. 15. Probability plots of faecal coliform counts (MPN) at different periods of depuration time for the experimental lots referred to under Group 3. ↓ indicates "less than"; ↑ indicates "more than".

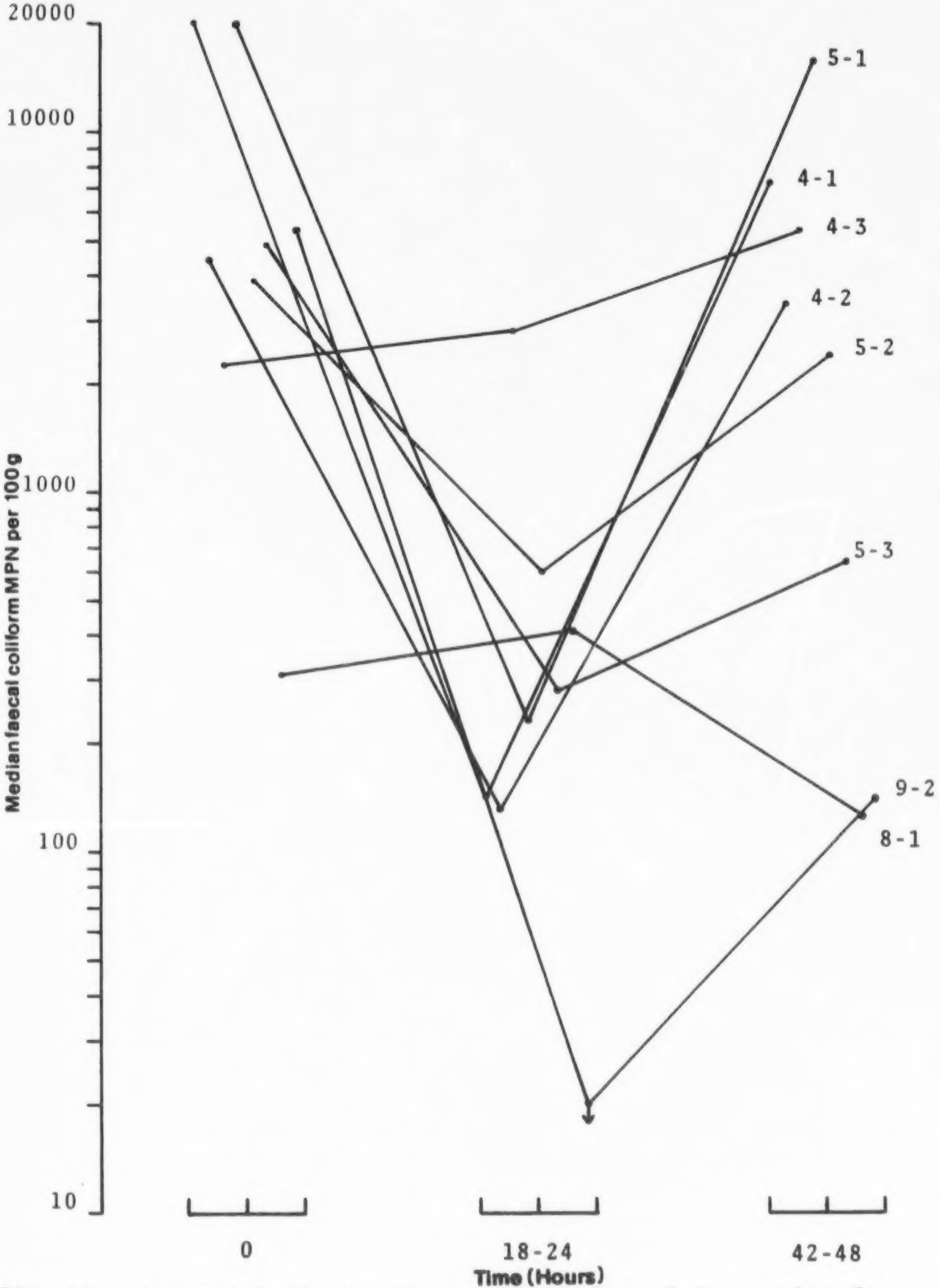


FIG. 16. Bacterial fluctuations versus time of depuration for the different experimental lots referred to under Group 3.

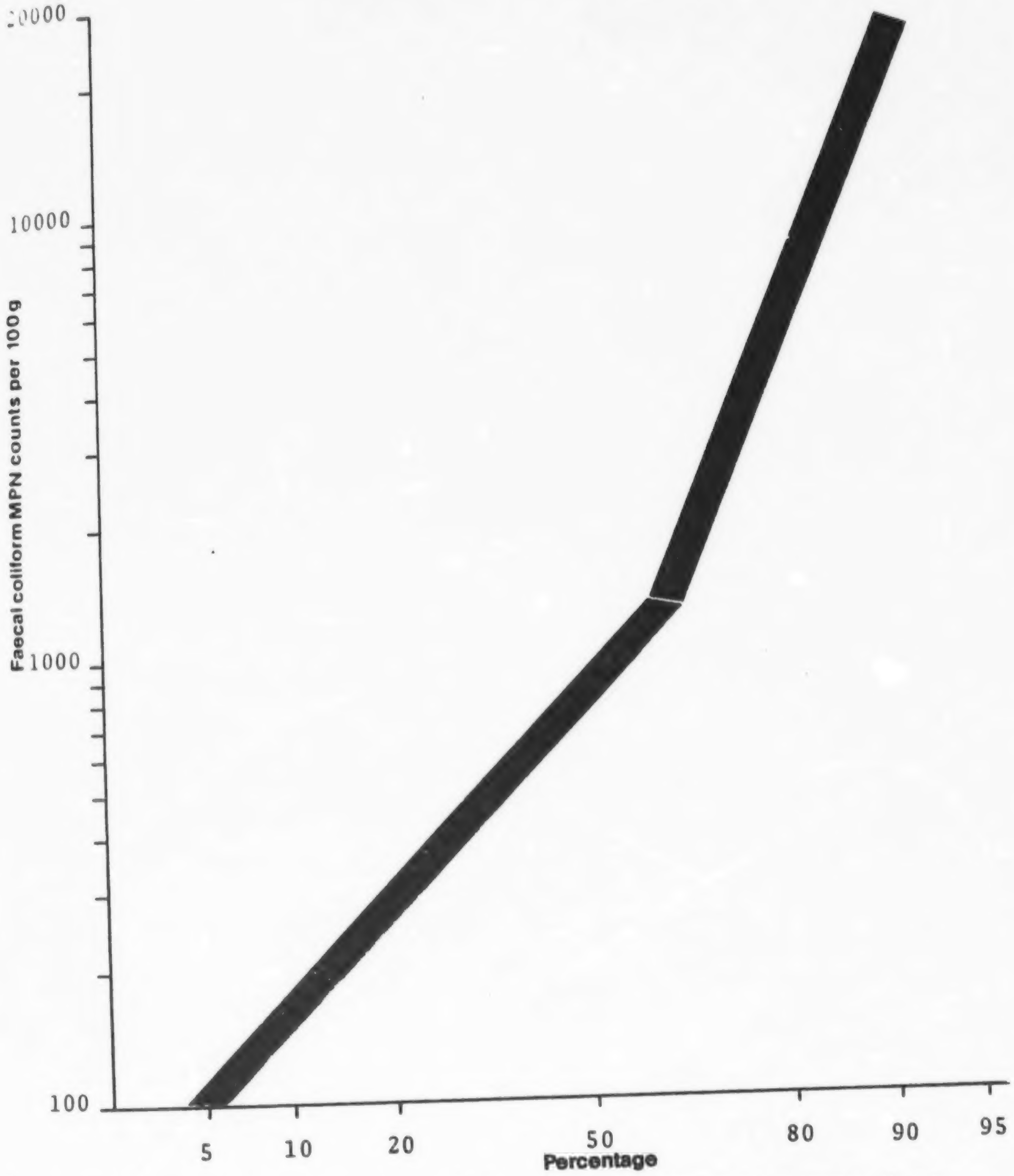


FIG. 17. Probability plot of faecal coliform MPN counts from shellstock samples and 0 Hour samples of the depuration trials.

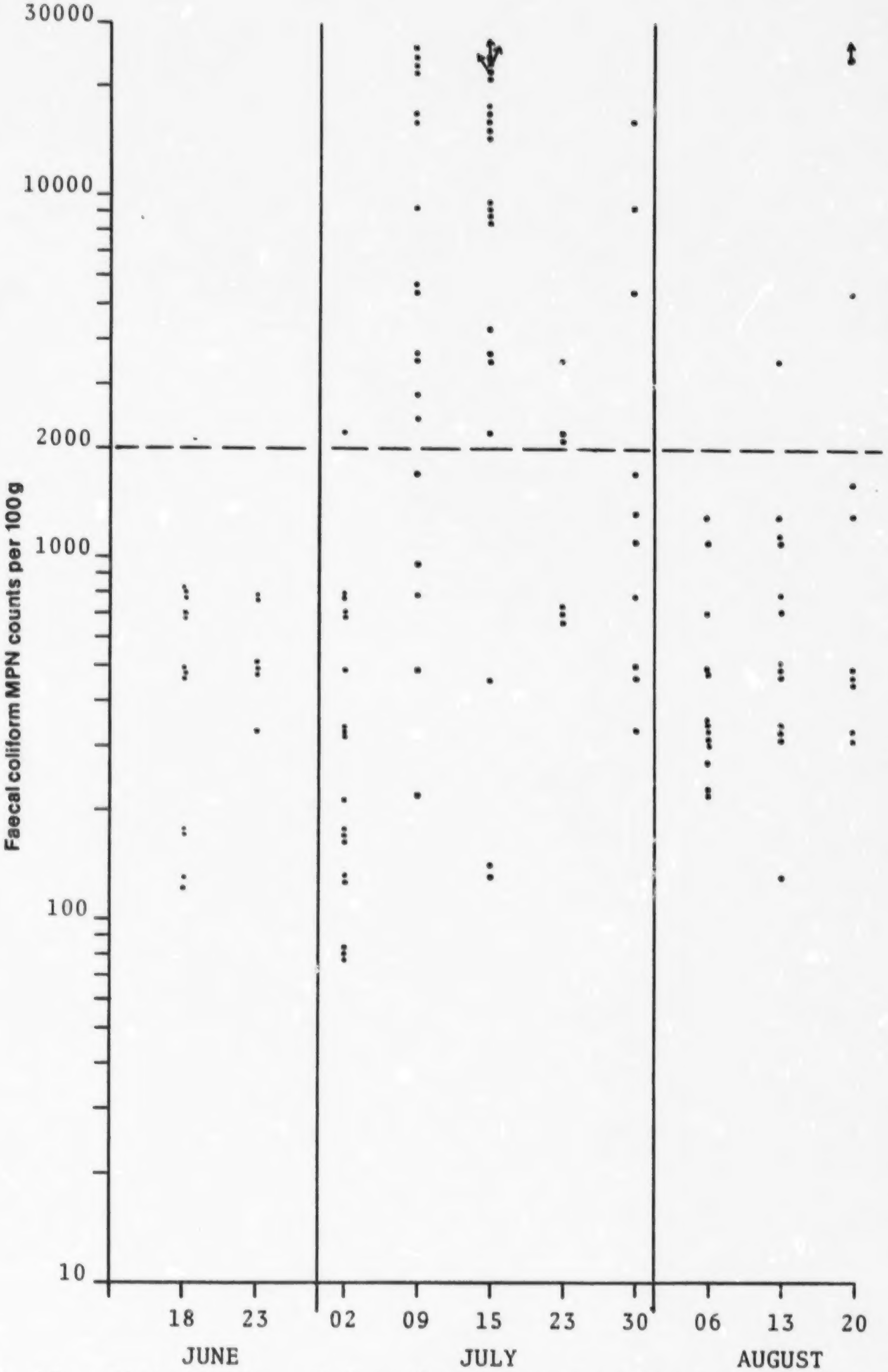


FIG. 18. Distribution of faecal coliform counts of shellstock an
0 Hour samples over the summer period when trials took place.
↑ indicates "more than".

APPENDIX A

COSTS OF DEPURATION

The costs of depuration are presented here as additional costs (required specifically because of the depuration process) to the existing cost of producing shucked clam meat. Costs are evaluated in terms of labour, equipment, building, services and an assumed loss to depuration of 5% of the clams purchased and processed.

For the purpose of these estimates, we assume a new room (depuration room) attached to an existing shucking plant. The room has one section for washing the clams and treating the water and another section for the depuration tanks.

We also assume a depuration system capacity of six tanks, each holding 21 bushels of clams (42 baskets per tank) for 42 hours. Tanks loaded Tuesday, Thursday and Saturday are emptied Thursday, Saturday and Monday. That is a maximum production of six tanks, three times a week (18 tank-loads). Since, it is unlikely the plant would operate at full capacity year round, we assume a maximum operational period of 25 weeks per year. Costs have been established for two, four or six tanks operating two or three times a week for 20 or 25 weeks per year.

Labour

Manpower and working time have been estimated from observations made during the depuration trials on runs with fully loaded tanks (three tanks of 42 baskets each). Culling the quantity of clams to load one tank is estimated to take two cullers 15 minutes (0.5 man-hour). The labour involved in filling the depuration baskets (two men) is not included in this costing, since (in normal shucking operations involving clams from uncontaminated areas) the clams are washed, placed in baskets and weighed. There is no additional cost. At the start of the depuration cycle, it takes two men half an hour to load one tank (take 42 baskets of clams from the weight-scale to the tank, place them in the tank and fill the tank with water); this is equal to one man-hour per tank. At the mid-point of the depuration cycle it takes two men about one hour to drain, unload, hose the clams and tank, reload and refill the tank again (two man-hours). At the end of the depuration cycle it takes two men about half an hour to drain, unload, wash the clams and move them to storage, and to do a general clean-up (one man-hour). Supervision of the operations, bacteriological sampling, and monitoring tests are performed by the foreman. He is also expected to take part in the labour operations.

Labour costs are computed at: (a) $3.00 per man-hour for the labourers, and (b) $4.00 per hour for the foreman.

	Man-hours
Culling	0.5
Start-point	1.0
Mid-point	2.0
End-point	1.0
	4.5 per tank

(a) $$\frac{3.5\ \text{man-hours}}{\text{tank}} \times \frac{\$3.00}{\text{man-hour}} = \$10.50$$

(b) $$\frac{1.0\ \text{man-hour}}{\text{tank}} \times \frac{\$4.00}{\text{man-hour}} = \$\ 4.00$$

$$\$10.50 + \$4.00 = \frac{\$14.50\ \text{labour}}{\text{tank (21 bu.)}}$$

$$\$14.50 \div 21\ \text{bu.} = \$0.69\ \text{per bushel for labour}$$

Labour cost per bushel is found to be constant whatever regime the depuration plant is operating under.

Equipment

Equipment costs, including a 10-year depreciation cost, are presented below. These figures assume the maximum capacity of six tanks. Although the depreciation cost is based on 10 years, some pieces of equipment will, of necessity, be replaced sooner while others will last longer. This cost is constant for the plant but varies from $2.05 down to $0.36/bushel (Table A-3), depending on the number of depurated bushels per year.

Pump, cable, frame	$ 4,200
Piping	2,000
Strainers and fittings	2,000
Sterilizer unit	1,000
Clam washer	7,000
Tanks (6)	7,000
Flow-meters (6)	8,600
Baskets and boxes	2,600
	$34,440
Installation	$ 3,000
10 years depreciation	$ 3,440/year.

Building

Building costs have been computed on the basis of the area required for a six-tank operation.

Depuration Area: 1,800 sq. ft. at $15.00 per sq. ft.
= $27,000.

This cost remains constant for the plant but varies from $0.80 to $0.14/bushel (Table A-3), depending on the number of bushels processed through the depuration plant per year.

Services

Maintenance and overhead costs include repairs on the pump and pipeline, painting, and office expenses. This is estimated at 5% of the capital cost of the equipment; some costs such as insurance are also involved whether or not the equipment is in use. This cost varies from $1.02 to $0.18 per bushel processed.

Assuming that the equipment may be used heavily, additional maintenance would be required (U.V. bulbs, wear on machinery, etc.). This is calculated as 5% of the labour costs and comes to $0.03 per bushel per year.

Electrical costs for the 16 H.P. pump is the most significant service cost. This cost is constant for any number of tanks used. The pump has to operate 50 hours per tank-loading. At $0.03 per kilowatt-hour, this comes to $17.90 per loading. Depending on the number of tanks used, two, four or six, this comes to $0.43, $0.21 and $0.14 per bushel per year.

Detailed computation of services:

a) 5% of capital cost of equipment ($34,400) = $1,720/year.

In a year, from 1,680 to 9,450 bushels may be processed, depending on the regime of operations:

$$\frac{\$1{,}720/\text{year}}{(1680,\ 2100,\ 2520...9450)\ \text{bu./year}} = \$1.02,\ \$0.82,\ \$0.68...\ \$0.18/\text{bushel}$$

b) 5% of labour cost

.05 x $0.69 = $0.03/bushel

c) Electrical costs

16 H.P. x 0.746 = 11.94 kilowatts

11.94 kilowatts x 50 hours = 596.8 kilowatt-hours

$$596.8\ \text{kilowatt-hours} \times \frac{\$0.03}{\text{kilowatt-hour}} = \$17.90/\text{tank-loading}$$

The cost of electricity per bushel has been computed (Table A-1) according to different tank-loadings per week and number of operating weeks per year.

The total service cost per bushel is presented in Table A-2.

TABLE A-2. Total services ($/bushel) according to different regimes of operation.

Tanks used	2				4				6			
Loads/week	2		3		2		3		2		3	
Weeks/year	20	25	20	25	20	25	20	25	20	25	20	25
Tanks/year	80	100	120	150	160	200	240	300	240	300	360	450
Bushels/year	1680	2100	2520	3150	3360	4200	5040	6300	5040	6300	7560	9450
a) $/bushel	1.02	.82	.68	.55	.51	.41	.34	.27	.34	.27	.23	.18
b) $/bushel	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03	.03
c) $/bushel	.43	.43	.43	.43	.21	.21	.21	.21	.14	.14	.14	.14
Total Services $/bushel	1.48	1.28	1.14	1.01	.75	.65	.58	.51	.51	.44	.40	.35

Loss in Undepurated Lots

In the best of conditions, it is possible that some tank-loads of clams will not depurate for unexplained reasons. During the 1975 depuration trials, three lots out of the 23 experimental lots could be classified as such. Two of these were, however, borderline, depurating well to the 18-24 Hour sampling and exhibiting only a slight increase at the 42-48 Hour sampling. All other undepurated lots could be explained in terms of experimental operating procedures, overly high initial bacterial loading, etc. Based on these results, our knowledge of losses experienced at presently operating plants and the probability that overall depuration success will be greater at other periods of the year, a 5% loss estimate has been used to cover the cost of lots that go through the depuration process, yet do not depurate successfully. The raw material and processing costs of these lots (cost of clams bought from the fishermen, labour and service costs involved) must be added to the costs of the lots which depurated satisfactorily.

Assuming a maximum production of 9,450 bushels/year or 450 tanks/year, a 5% loss would amount to 23 tanks or 483 bushels.

Cost of undepurated clams: 60 lbs/bu. x $0.17/lbs = $10.20/bu.

$10.20/bu. x 483 bu. = $4,926.60

This cost of lost clams has to be accounted for in the cost of clam lots that will depurate (450 - 23 = 427 tanks or 8,967 bushels).

Cost of undepurated clams/bu. of depurated clams:

$$\frac{\$4,926.60}{8,967 \text{ bu.}} = \$0.55/\text{bu.}$$

Labour: Costs for labour performed on lost clams is also charged to the costs of depurated clams.

$0.69/bu. x 483 bu. lost clams = $333.27

This cost spread over 8,967 depurated bushels comes to $0.04 per bushel.

TABLE A-1. Cost of electricity per bushel according to different tank-loadings per week and number of operating weeks per year.

Tanks used	2				4				6			
Loads/week	2		3		2		3		2		3	
$/week	35.81		53.71		35.81		53.71		35.81		53.71	
Weeks/year	20	25	20	25	20	25	20	25	20	25	20	25
$/year	716.20	895.25	1074.20	1342.75	716.20	895.25	1074.20	1342.75	716.20	895.25	1074.20	1342.75
Bushels/year	1680	2100	2500	3150	3360	4200	5040	6300	5040	6300	7560	9450
$/bushel	0.43	0.43	0.43	0.43	0.21	0.21	0.21	0.21	0.14	0.14	0.14	0.14

Services: Assuming maximum production, service costs are $0.35/bu.

$0.35/bu. x 483 bu. lost clams = $169.05

Spread over 8,967 bu. the extra service costs add up to $0.02/bu.

Total costs due to a 5% loss to depuration per bushel:

costs of clams	$0.55
labour	0.04
services	0.02
	$0.61

This cost is approximately constant whatever the depuration production is. For a minimum production of 1,680 bu./yr. (two tanks twice a week for 20 weeks), it is $0.65/bu.

Total Costs

Total costs are presented in Table A-3. Depending on the operating schedule of the depuration plant and the different items included in the costing, the cost of depurating a bushel of clams varies from $5.67 to $2.15. If only labour and services are considered, the cost of depurating a bushel of clams varies from $2.07 to $1.04. Adding a 5% cost for labour and services on lots which fail to depurate, increases these costs to $2.72 and $1.65. This 5% loss could be reduced by improvements to the physical plant and operating procedures and by restriction of the harvest site to even more marginally polluted areas.

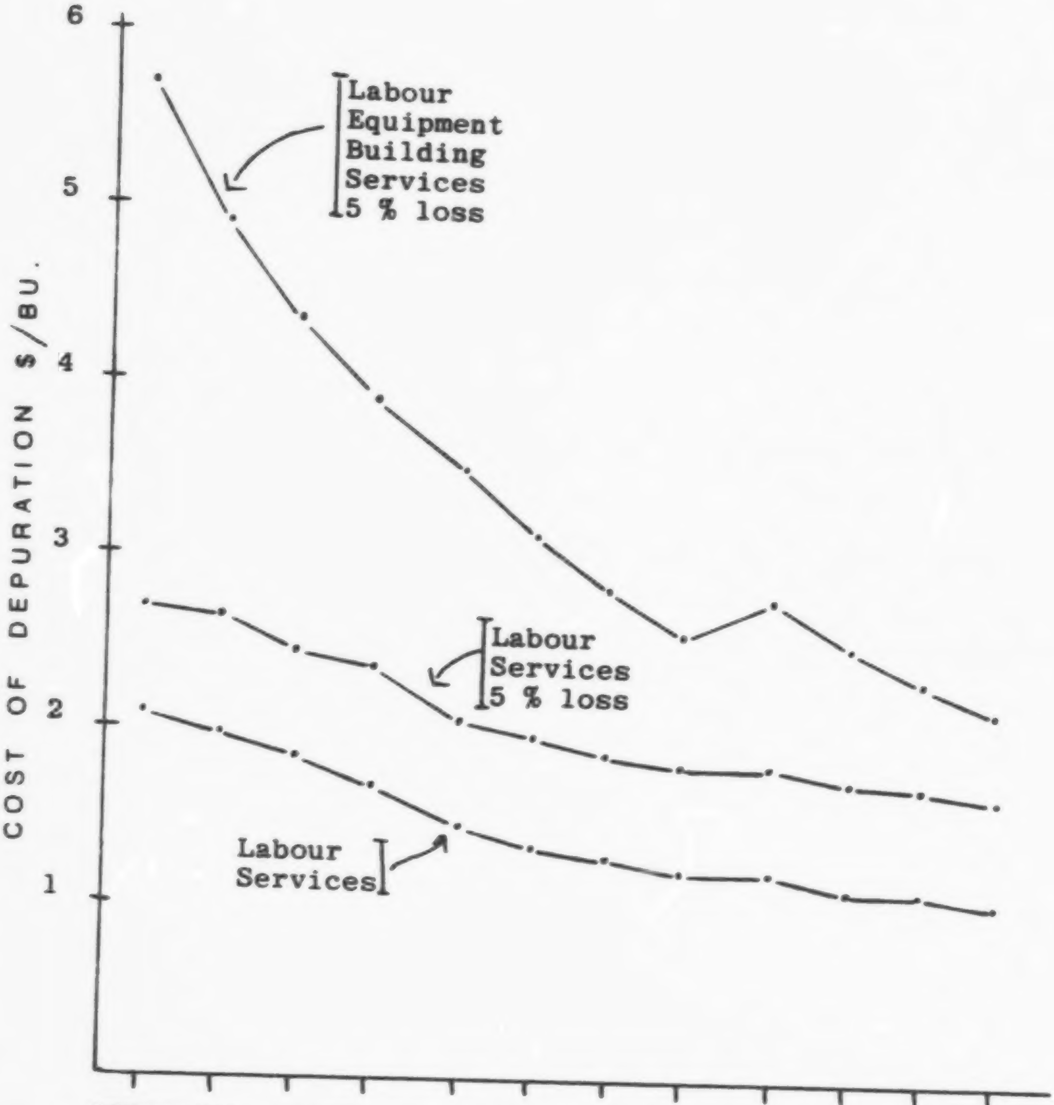
These costs, presented graphically in Figure A-1, do not include costs for weighing, washing, basket loading, or culling losses since these costs are incurred in any clam processing operation.

TABLE A-3. **Costs for depuration depending on the operating schedule of a depuration plant with 6 tanks.**

Tanks used	2				4				6			
Loads/week	2		3		2		3		2		3	
Weeks/year	20	25	20	25	20	25	20	25	20	25	20	25
Tanks/year	80	100	120	150	160	200	240	300	240	300	360	450
Bushels/year	1680	2100	2520	3150	3360	4200	5040	6300	5040	6300	7560	9540

$ per depurated bushel of clams

1) Labour	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69	.69
2) Equipment	2.05	1.64	1.37	1.09	1.02	.82	.68	.55	.68	.55	.46	.36
3) Building	.80	.64	.54	.43	.40	.32	.27	.21	.27	.21	.18	.14
4) Services	1.48	1.28	1.14	1.01	.75	.65	.58	.51	.51	.44	.40	.35
5) 5% loss	.65	.64	.63	.67	.61	.61	.58	.60	.60	.60	.59	.61
1) & 4)	2.07	1.97	1.83	1.70	1.44	1.34	1.27	1.20	1.20	1.13	1.09	1.04
1) & 4) & 5)	2.72	2.61	2.46	2.37	2.05	1.95	1.85	1.80	1.80	1.73	1.68	1.65
1) to 5) incl.	5.67	4.89	4.37	3.89	3.47	3.09	2.80	2.56	2.75	2.49	2.32	2.15



Tanks used	2				4				6			
Loads/week	2		3		2		3		2		3	
Weeks/year	20	25	20	25	20	25	20	25	20	25	20	25
Tanks/year	80	100	120	150	160	200	240	300	240	300	360	450
Bu./year	1680	2100	2520	3150	3360	4200	5040	6300	5040	6300	7560	9540

FIG. A-1. Cost of depuration ($/bushel).

APPENDIX B

EQUIPMENT AND DESIGN

Equipment

Depuration Tank Capacity

3 tanks.

Maximum of 21 bushels per tank.

Maximum of 3 layers of baskets per tank (42 baskets).

½ bushel (30 pounds) of clams per basket.

Seawater Delivery and Treatment

Pump: 16 H.P. submersible Pleuger N-85; 575 Volts, 3 ohms, 60 Hertz, 3450 RPM, 150 gpm. @ 200 feet head connected to 2100 feet #6 AWG Cable.

Screening and Filtration:

1. Pump-frame mounted: 2-4 feet x 4 feet screens; ½-inch mesh.
2. Pump mounted: 5 inches diameter plastic screen, 5/16-inch mesh, 4 inches long.
3. In-line strainer: Zurn, 6 inches x 1/16-inch (not used).
4. In-line filter: Cuno 150-millimeter cartridge (not used).

Sterilizer: 10 30-watt Ultra-Violet (2537 angstroms) germicidal lamps. General Electric G 30 T 8, total length of 36 inches, effective length of 32 inches, 7500 hours, average output 6.6 watts. The lamps were mounted 9 inches above the water on 3 3/4-inch spaces.

10 ballasts American Ultra Violet No. 202 B, Universal Thermomatic preheat lamp ballast, cat. no. 202-B-TC-P.

10 starters FS-4.

20 ends Med. Bipin.

1 switch.

10 pilot lights.

Flow-meters: 30° V-notch weir recorded on a Fisher-Porter 7-day float actuated recorder. Meter No. 10F1272A1102 AA02.

Clam Treatment

Conveyor-washer: Stainless steel construction. Dimensions (feet) - 17 x 1 x 4 high, ½-inch mesh conveyor belt (24 feet per minute) with 8 water sprays directed downward and 4 up.

Basket Filling Tank: Fiberglass reinforced plastic. Dimensions (feet) - 6 x 1½ x 1 deep with overflow and drain.

Depuration Baskets: ½-inch square mesh 17/19 gauge green PVC coated wire. Dimensions (inches) - 11 x 21 5/8 x 6 3/4 high. Volume: 1573 cubic inches, or 0.71 bushel. 71% open area through mesh.

Weigh Scale: Floor model Howe-Richardson scale, Model 54-3, 1000 lbs. capacity.

Depuration Tanks: White fiberglass reinforced plastic. Dimensions (feet) - 4 x 8 x 3 deep. Drains - 3-inch threaded, at outlet end of tank; - 2-inch stoppered, on bottom of tank. 3 skim holes 1½-inch in diameter at outlet end of tank, 2 inches from top of tank. Baffle located 8 inches from the inlet end, extending from the top to 2 inches from the bottom of the tank. 2-inch spacers of PVC pipe.

Design Criteria

Pump: The pump was sized for 150 gpm (Imp.) to provide an adequate water flow for a full scale plant capacity (water flow of one gallon per minute per bushel for six tanks holding 21 bushels of clams with additional capacity for washing). The vertical distance from the surface of the water at low tide to the plant was 65 feet. The discharge was estimated at 30 psi gauge pressure (71 feet head) to allow for the strainer and filter. At 150 gpm, the pipe friction was 6.5 feet drop per 100 feet of pipe for a 3-inch diameter 100 psi gauge pressure pipe (inside diameter: 2.8 inches). (Dupont of Canada "Sclair-pipe polyethylene pipe for engineered systems" catalog p. 30). Total friction was 130 feet. Total head for the submersed pump was estimated at:

discharge pressure	46 feet
friction	130 feet
elevation	+ 45 feet
Head	221 feet

The submersible pump was a type N85-3 + V8-40 for 8-inch diameter wells. The pump had its motor filled with fresh water before being put in place.

Electrical power was connected to the pump from a 600-Volt source 2100 feet away through no. 6 size copper wire laid on the bottom. The splice near the pump was made as outlined in the Pleuger catalog (Pleuger of Canada Limited. "Submersible pumps" catalog, section 6, p. 137, August 1971, Cooksville, Ontario). The splicing had to be redone due to a leak in its insulation. The splice repair was made using a Scotchcast communications splicing kit (no. 89-D3).

The motor starter was supplied by Westinghouse: AC magnetic starter cat. no. A200S2CE, Style 276A 621605, size 2, poles 3, with enclosure CEMA 1. The coil was powered from 550 Volts to start the motor after a power failure. Three heaters were used. The pipe line was buried from the plant out to the low water mark. Three steel drums were used as float markers to identify the location of the pipe, electrical lines, and the pump. The pump was submersed in 8 to 10 feet of water at low tide.

Sterilizer: A sterilizer similar to the Kelly-Purdy type was used because it was inexpensive, easily cleaned and inspected.

The sterilizer box was made of plywood (½-inch thick) coated with fiberglass reinforced plastic. Drain holes were plugged with rubber stoppers. Inlet water was distributed through PVC pipes. Lamps were held in brackets attached to the inside of the top of the box. Wires were connected to the brackets through holes drilled in the top of the box. A section was added to the box in the eventuality that a fan would be required to ventilate the bulbs; this was not required. There was also an opening near the water inlet to let the ventilation air out. Aluminum foil was attached to the top of the box behind the bulbs to increase light reflection.

Flow-meter Box: The flow-meter boxes were made from white polyethylene boxes by putting in partitions and drain holes (to fit a 3-inch pipe). Each box was 26 1/8 inches long and 16 inches wide. Information from Permutit ("Water and waste treatment data book", 1961, 9 ed., Paramus, N.J., p. 29) on "Drain pipes carrying capacities" suggested 25 gpm (Imp.) for a 3-inch pipe at a slope of 1.2 inch in 10 feet and 30 gpm (Imp.) for 1.6 inch in 10 feet. The flow-meter box was raised 6 inches. Automatic flow recorders were installed, however, missing and improper components prevented their use. Future installations should have either a deeper flow-meter float well or a smaller float ball since flow-rates smaller than 8 gpm could not be recorded as the float ball rested on the bottom of the flow-meter box. A length of pipe was added to the inlet valve (1½-inch) to extend it below the water surface and smoothen the flow over the weir. The V-notch in the flow-meter box was made to be a 30° angle with a height of 5 5/8 inches (0.469 feet). The theoretical flow "Q" for a height "h" over a weir of 30° is calculated according to Marks (Marks, L.S. ed. "Mechanical Engineers" Handbook, 5 ed., 1951, McGraw-Hill, N.Y., P. 245).

$Q = C\ 8/15\ \text{Tan}\ (\alpha/2)\ h^2\ \sqrt{2\ gh}$

Where Q = volumetric flow (cubic feet/second)

C = 0.59 constant for 30° weir

α = weir angle

g - gravity 32.2 feet/second^2

h = water height over the weir (feet)

$$Q = 0.59 \times 8/15\ \text{Tan}\ (30^0/2)\ h^2 \sqrt{\frac{2 \times 32.2\ \text{ft}}{\text{sec}^2} \times h}$$

$Q = 253\ h^2\ \sqrt{h}$ gal. per min.

using 0.469 feet as h

$Q = 253 \times (.469)^2 \times \sqrt{.469}$

= 38.13 gal. per min. as theoretical flow.

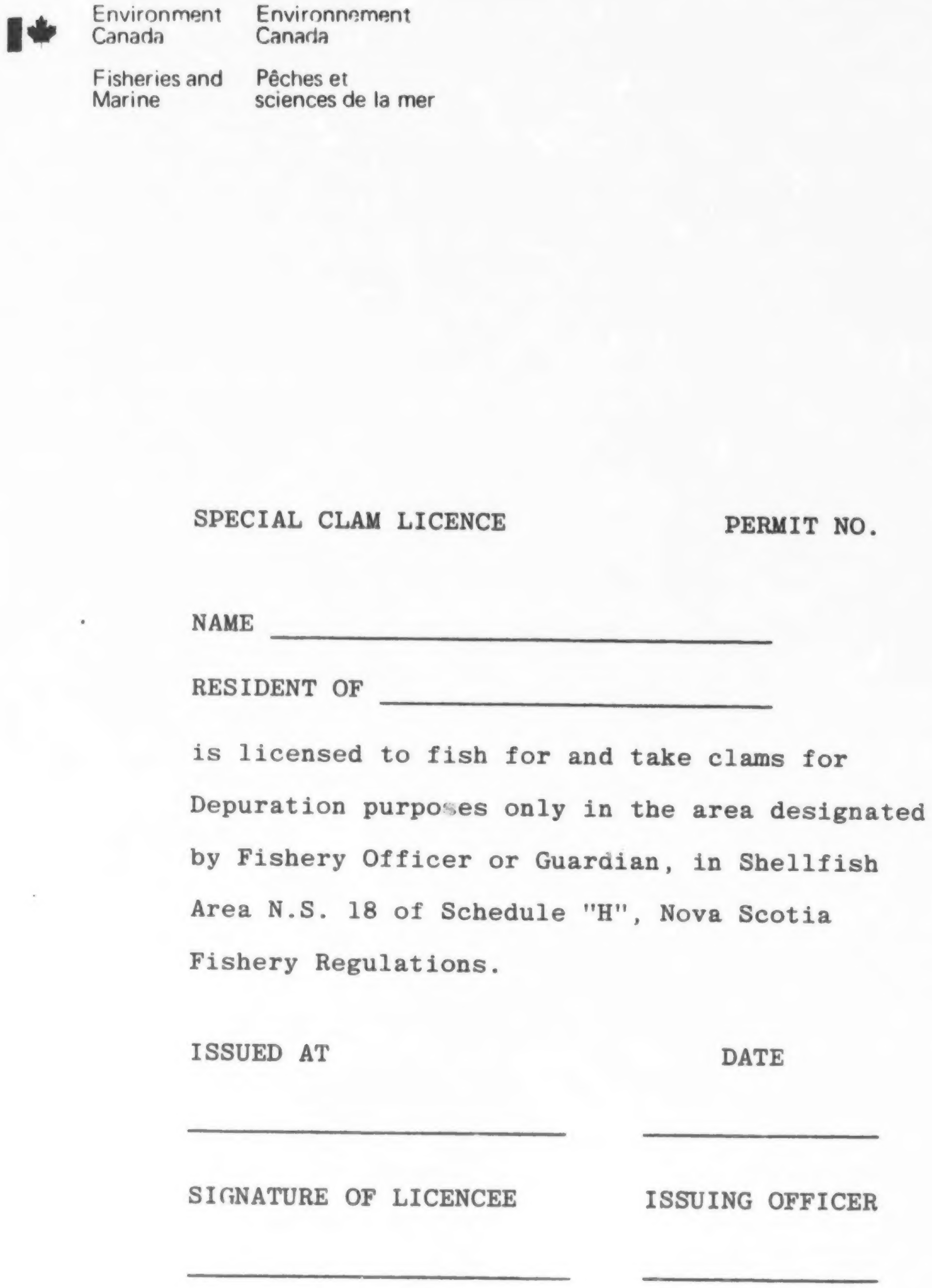
APPENDIX C

RECORDING MATERIALS

This appendix presents sample copies of a harvesting permit (Fig. C-1) and shellfish tag (Fig. C-2) used to identify the shellstock for depuration purposes. The tag was attached to the onion bag in which clams were carried. Seals were also used on the truck carrying clams from the harvest site to the depuration plant. These were of the metal band type similar to those used for general transport purposes.

The Fishery Officer or Guardian on duty at the harvest site was requested to record pertinent data while digging was taking place. Figure C-3 is an example of the shellstock harvesting report used.

A data recording sheet (Fig. C-4) was used to monitor physical, chemical, and bacteriological variables at the plant site.



Environment Canada   Environnement Canada

Fisheries and Marine   Pêches et sciences de la mer

SPECIAL CLAM LICENCE   PERMIT NO.

NAME ______________________

RESIDENT OF ______________________

is licensed to fish for and take clams for Depuration purposes only in the area designated by Fishery Officer or Guardian, in Shellfish Area N.S. 18 of Schedule "H", Nova Scotia Fishery Regulations.

ISSUED AT   DATE

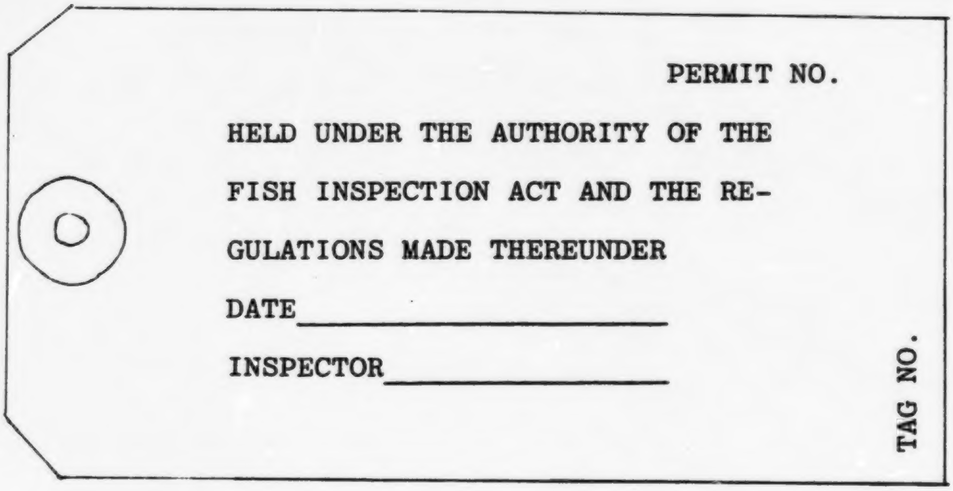
______________________   ______________

SIGNATURE OF LICENCEE   ISSUING OFFICER

______________________   ______________

FIG. C-1. Sample of a harvesting permit.

FRONT



PERMIT NO.

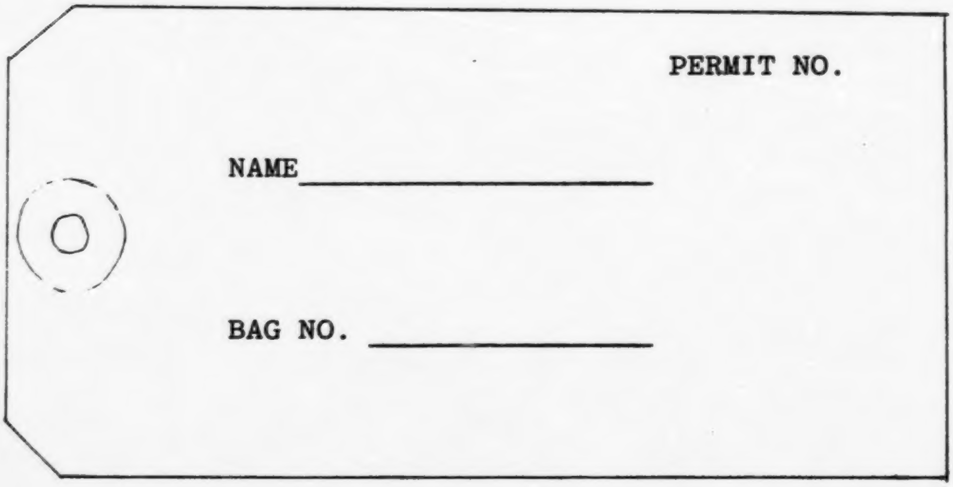
HELD UNDER THE AUTHORITY OF THE FISH INSPECTION ACT AND THE REGULATIONS MADE THEREUNDER

DATE____________________

INSPECTOR________________

TAG NO.

BACK



PERMIT NO.

NAME____________________

BAG NO. ________________

FIG. C-2. Sample of a shellfish tag.

PILOT DEPURATION PROJECT DIGBY N.S.

SHELLSTOCK HARVESTING REPORT

Date	Harvest location	Permit no. of diggers	Quantity harvested (lbs)	Weather conditions	Harvesting commenced	Harvesting completed	Lot no.	Known variables

Time of arrival at plant	Salinity	Water temperature	Remarks

Signature____________________

Title____________________

FIG. C-3. Sample of a shellstock harvesting report.

PILOT DEPURATION PROJECT DIGBY N.S.
LABORATORY ANALYSIS SUMMARY SHEET

Lab. No.	Type of sample	Time Date sampled	Sampling location	Faecal coliform per 100ml/ 100g	pH	Temp C	Salinity	Diss O_2 mg/l	Turbidity J.T.U.

FIG. C-4. Sample of data recording sheet used at the plant site.

APPENDIX D

RAW BACTERIOLOGICAL DATA

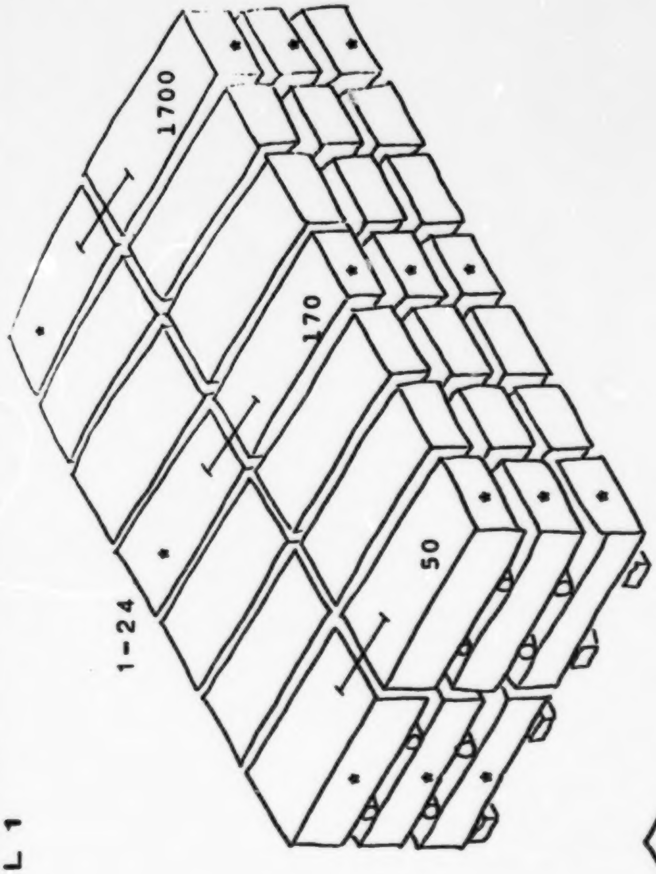
This appendix gives the detailed bacteriological counts for each of the trials and the disposition of the baskets sampled as they were loaded in the depuration tank during these trials. The number shown in a basket represents the bacteriological count (MPN Faecal Coliform per 100g clam meat) of a sample of clams taken from that specific basket or of a composite sample from those baskets marked by an asterisk (*). If a bridge (⊢—⊣) links two basket-stacks, this means that the bacteriological count shown is the result of a composite sample of clams coming from all baskets marked by an asterisk in those stacks.

The group of numbers on the outside of a series of baskets indicates first the tank number, second the period of depuration time, in hours, for which the samples were taken; 1-18 means tank 1 at 18 hours of depuration time for that particular trial.

For all trials the flow pattern in the depuration tank was from left to right.

TRIAL 1

1-24



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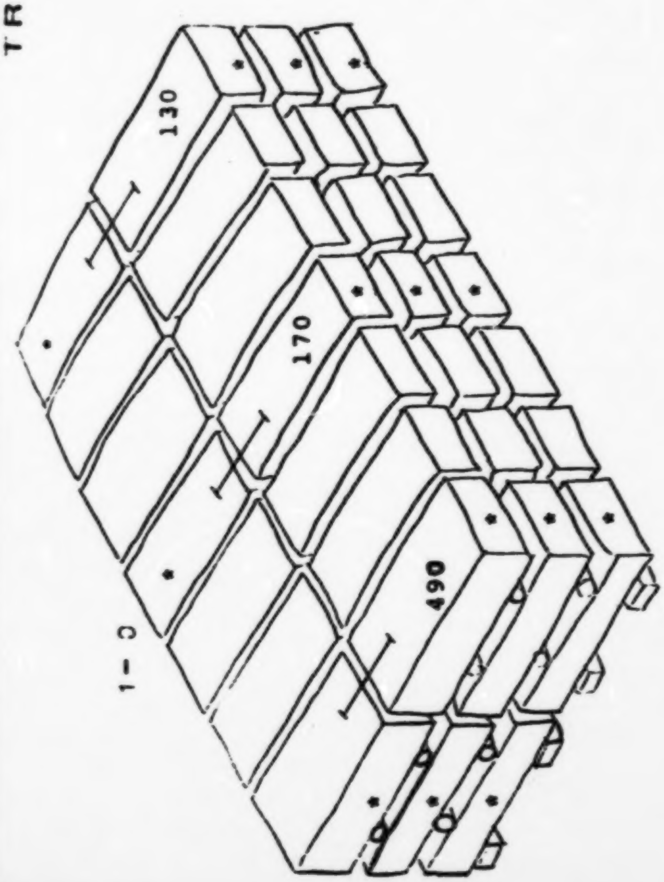

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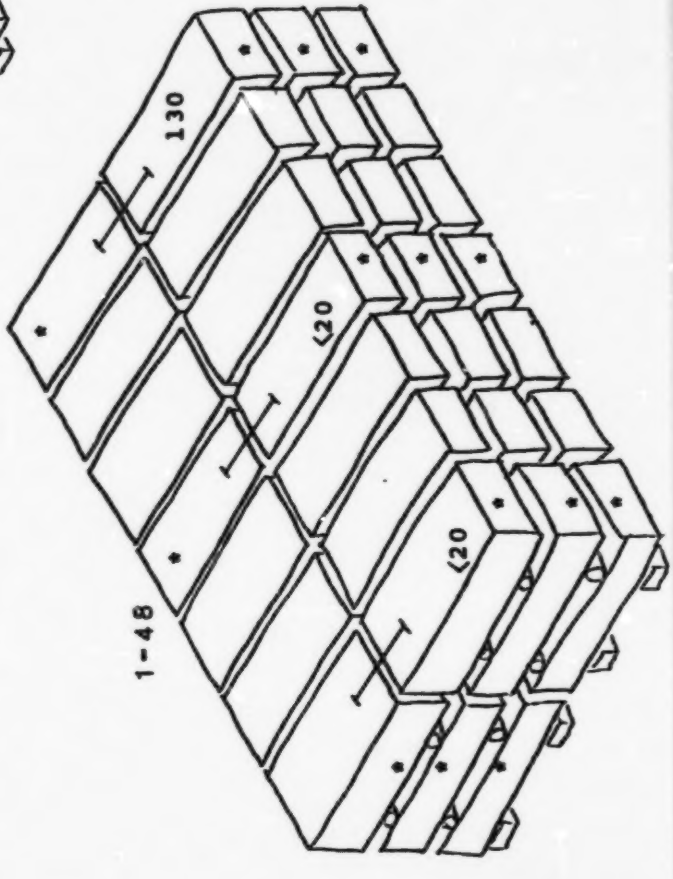
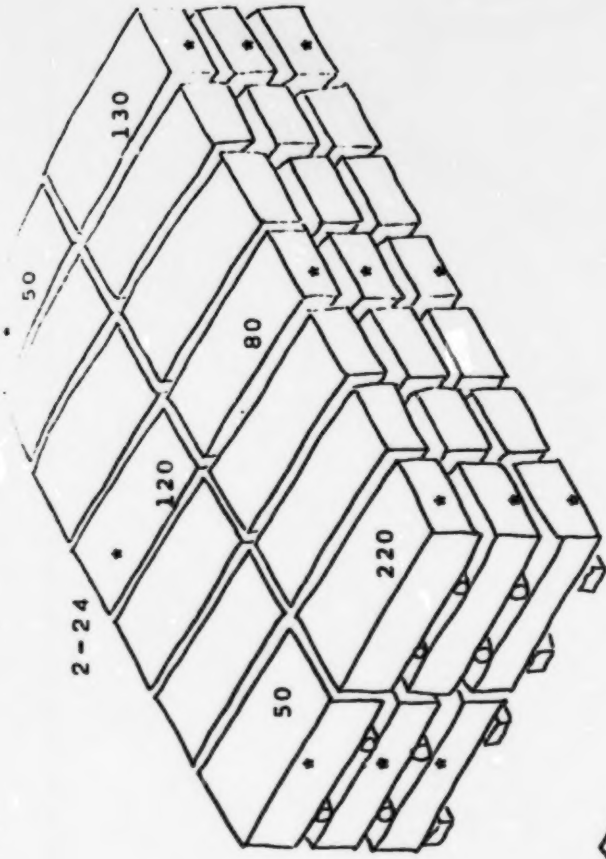
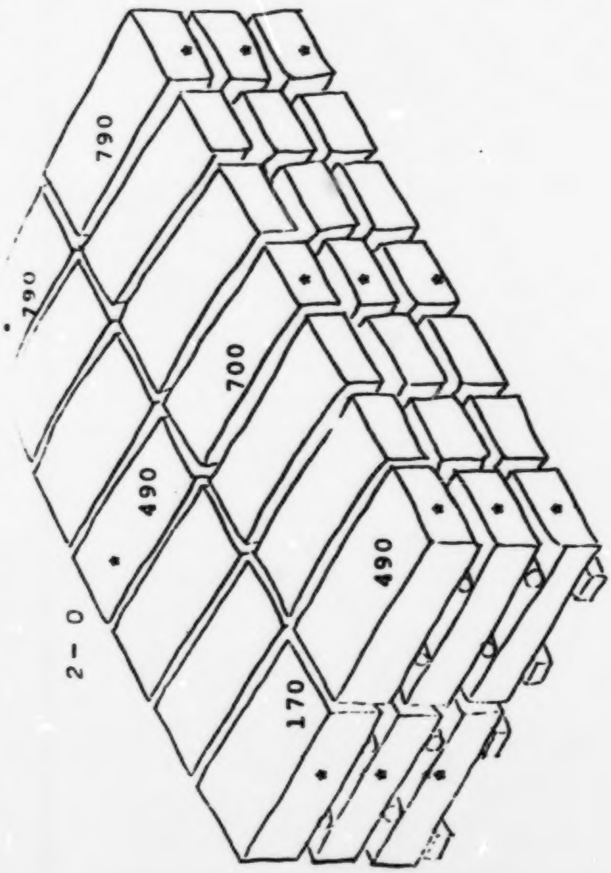




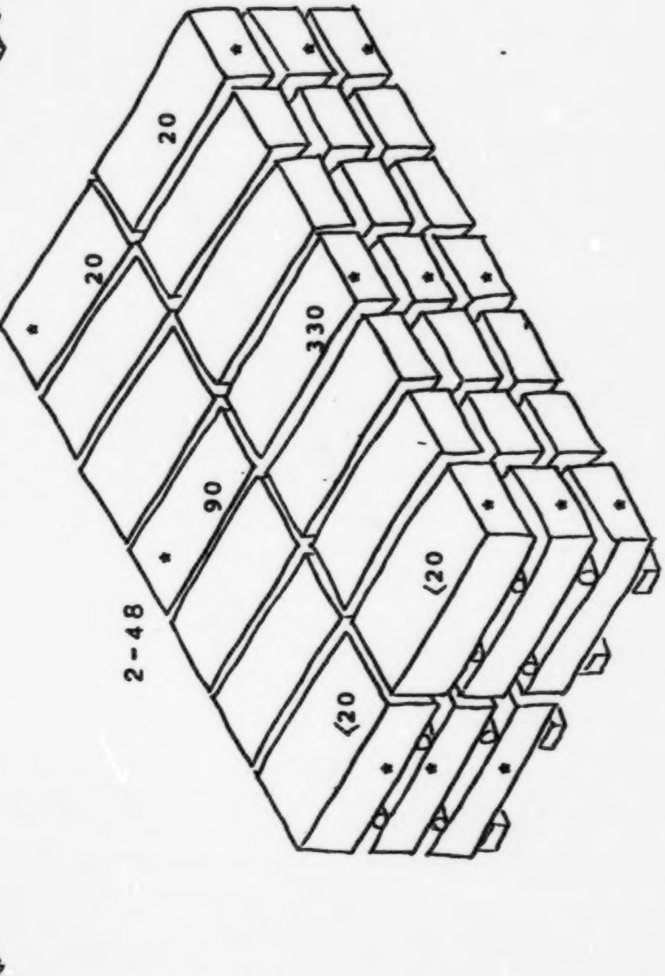
2-24

50
130
80
120
220
50



2- 0

790
790
700
490
490
170



2-48

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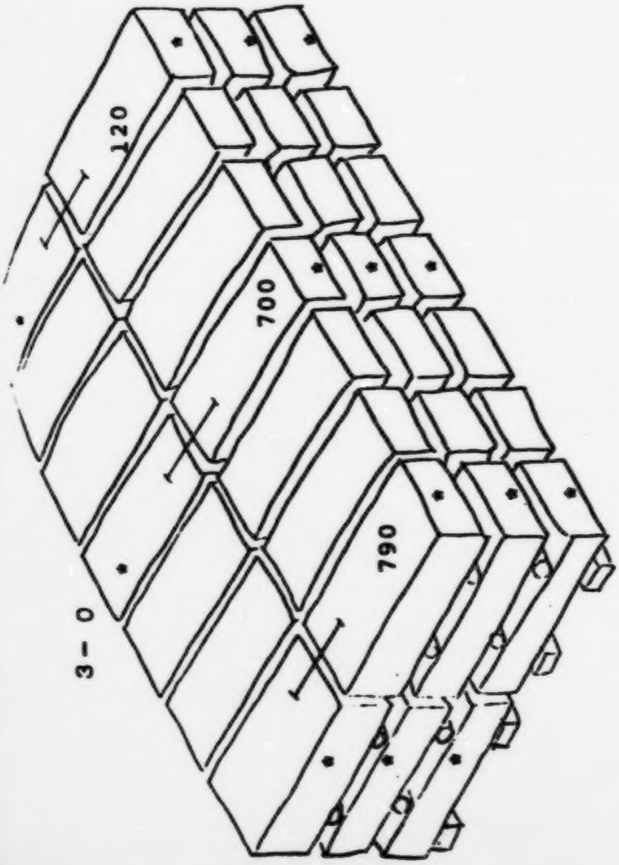
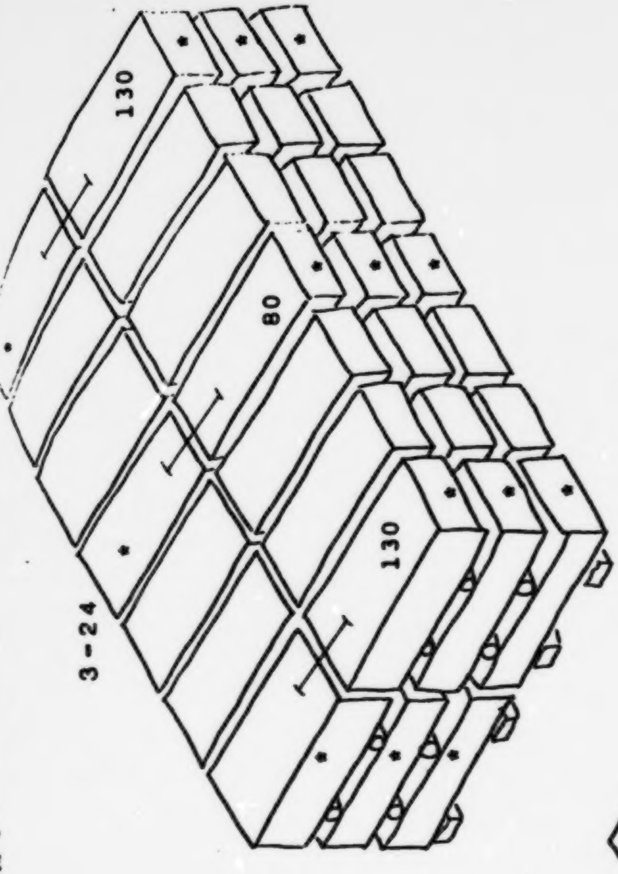
20
330
90
<20
<20



3- 0



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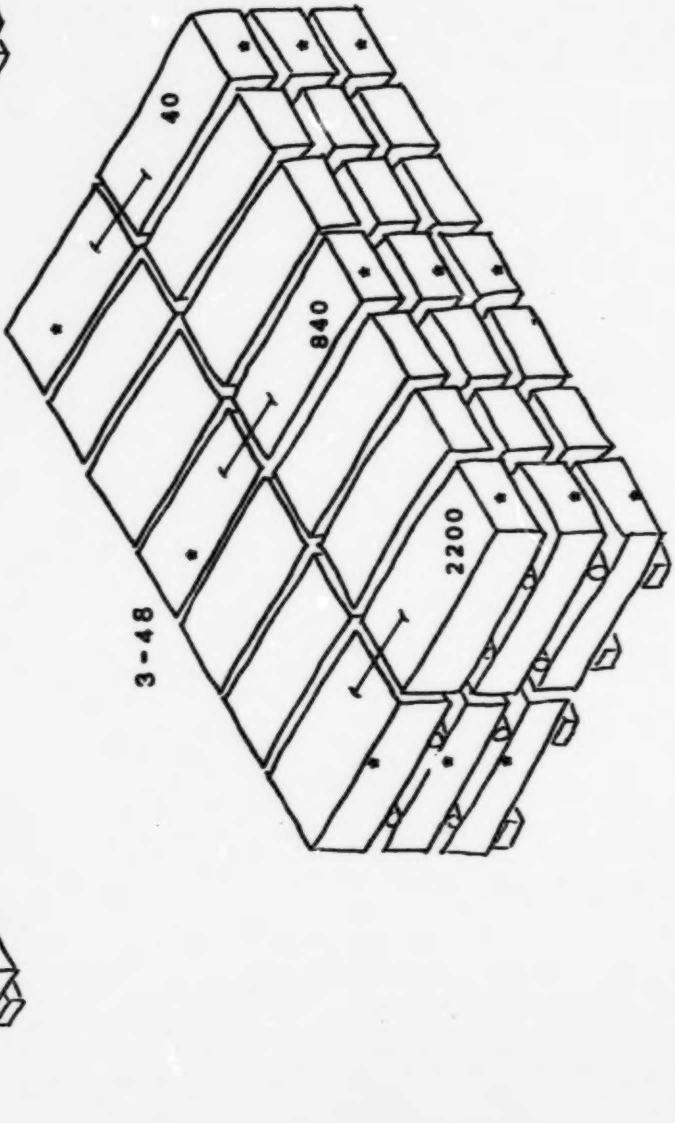

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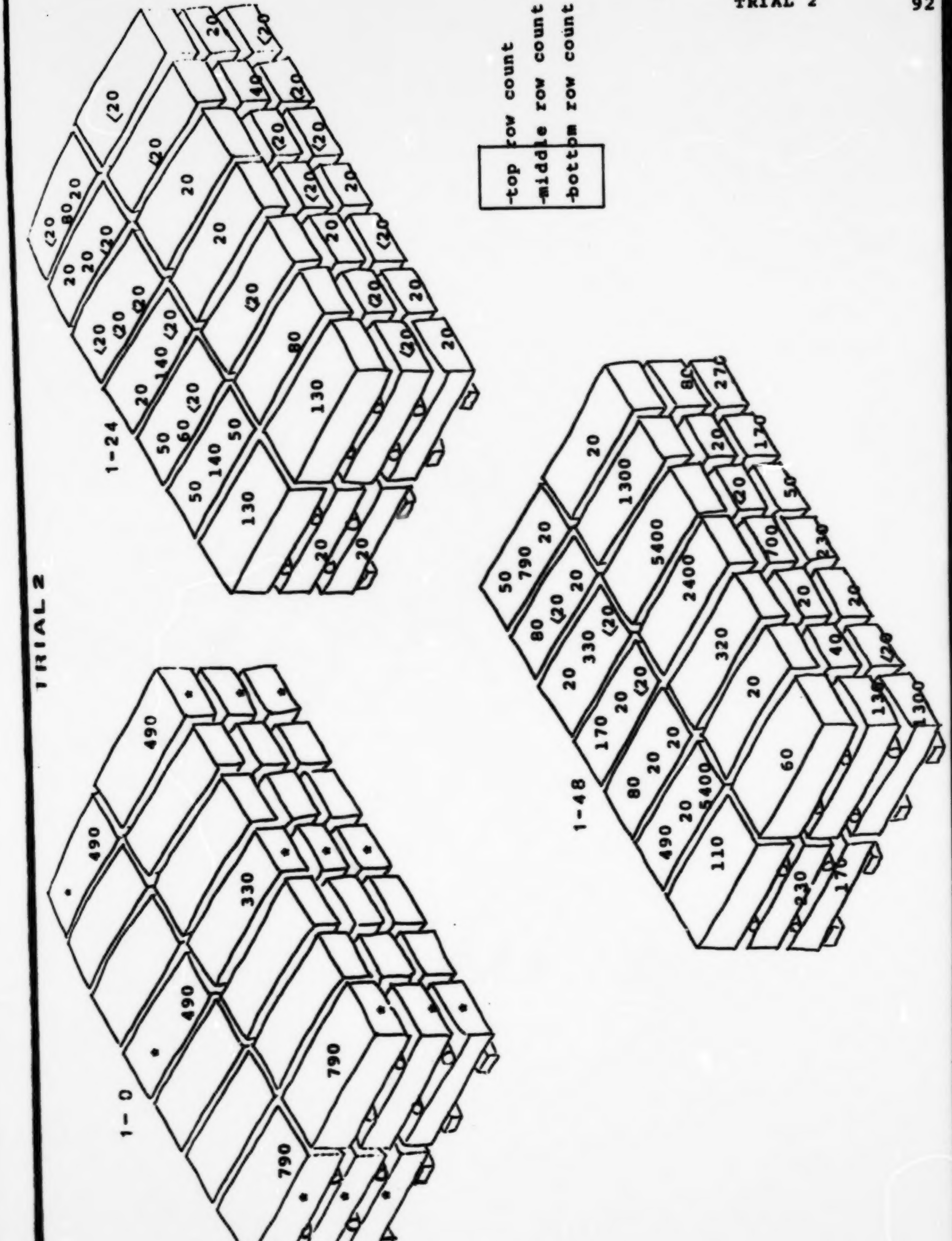
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1-48
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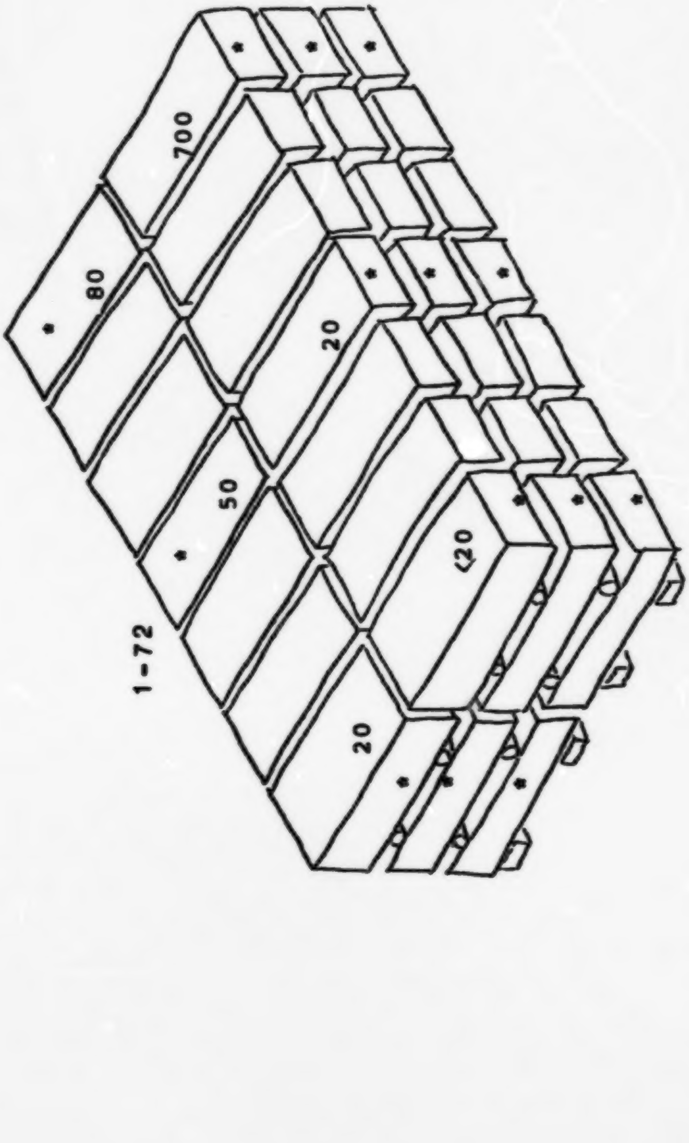
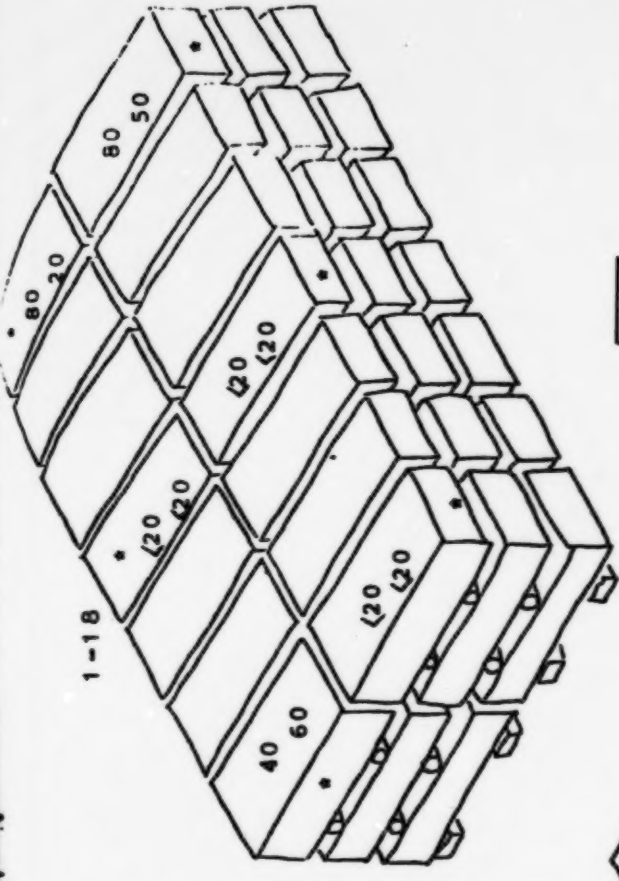
-middle row count
-bottom row count
<20
*


1-72
<20

count from top of basket

count from bottom of basket

1-18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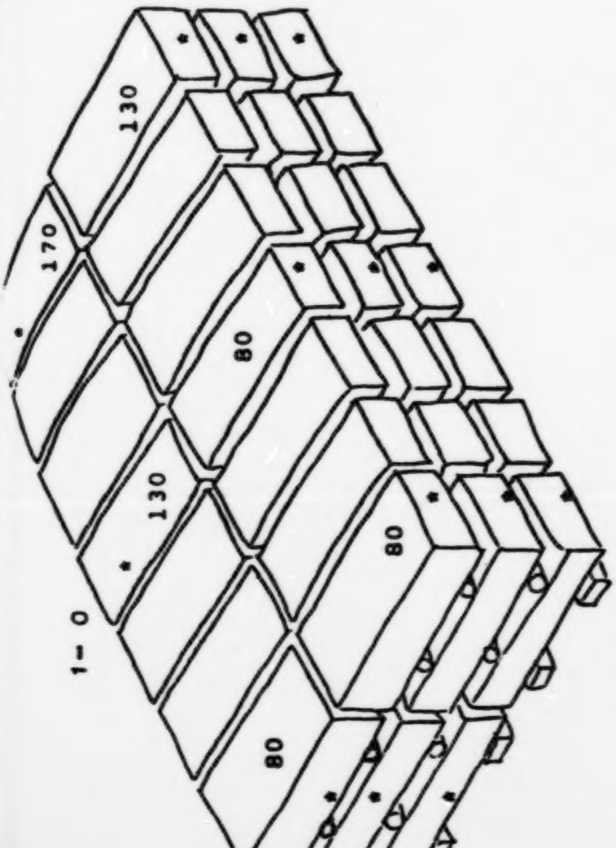
<20
<20
40
60

1-0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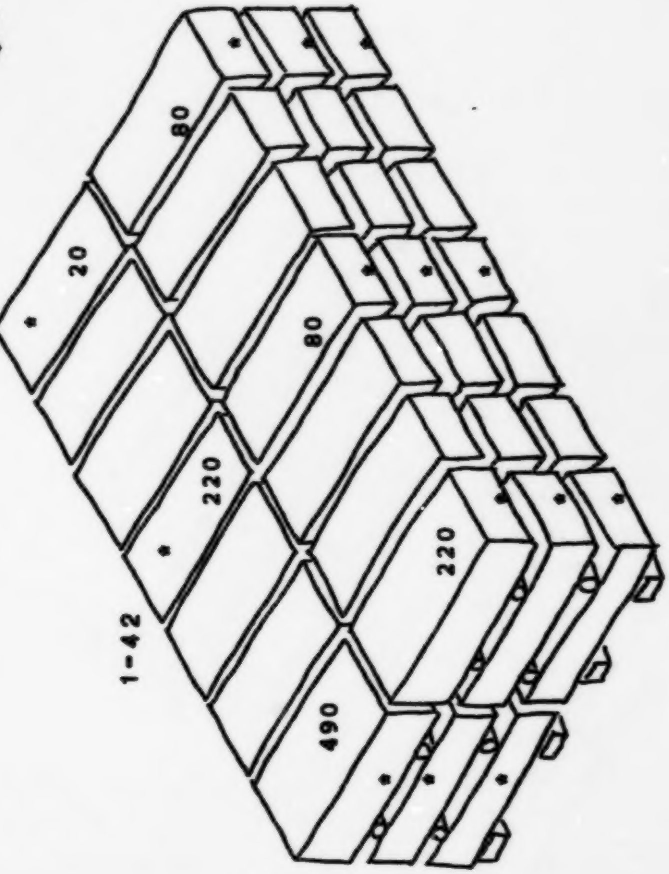
80
130
80
80

1-42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80
220
220
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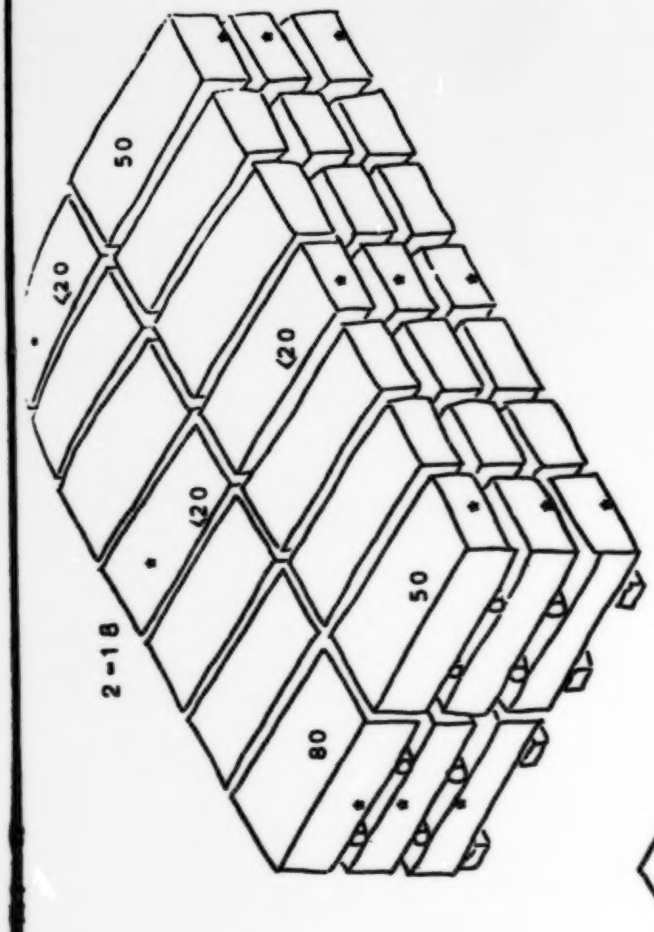
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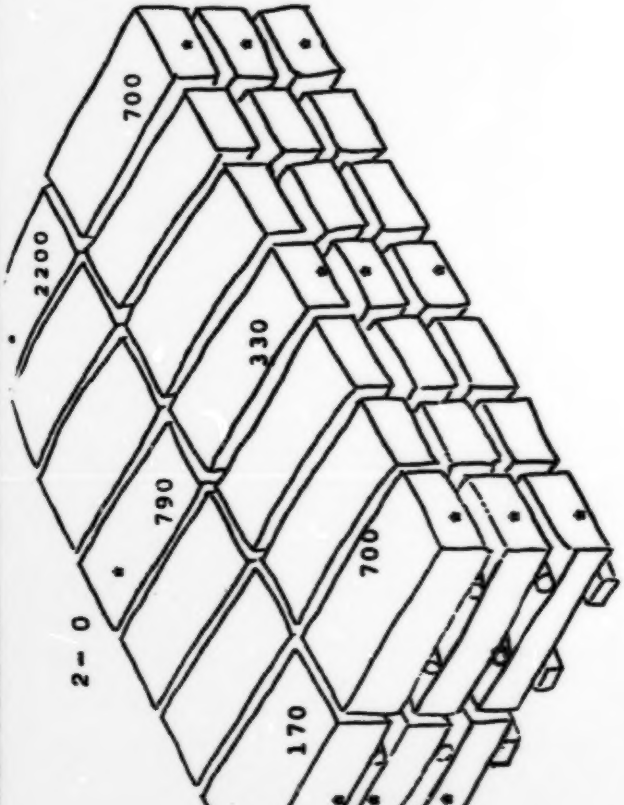
120
120
50
80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2200
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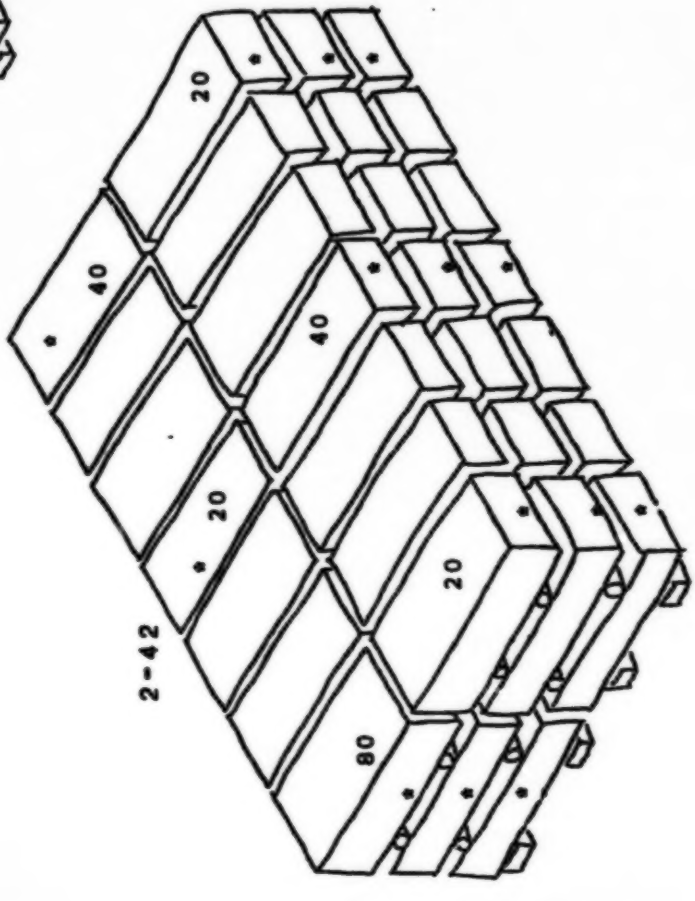
790
700
170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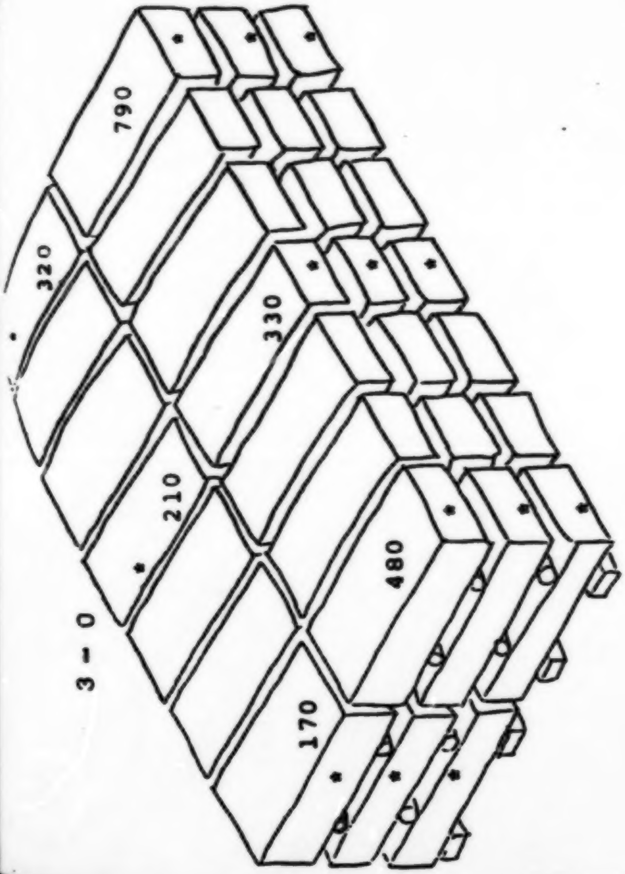
40
20
20
80

3 - 0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210
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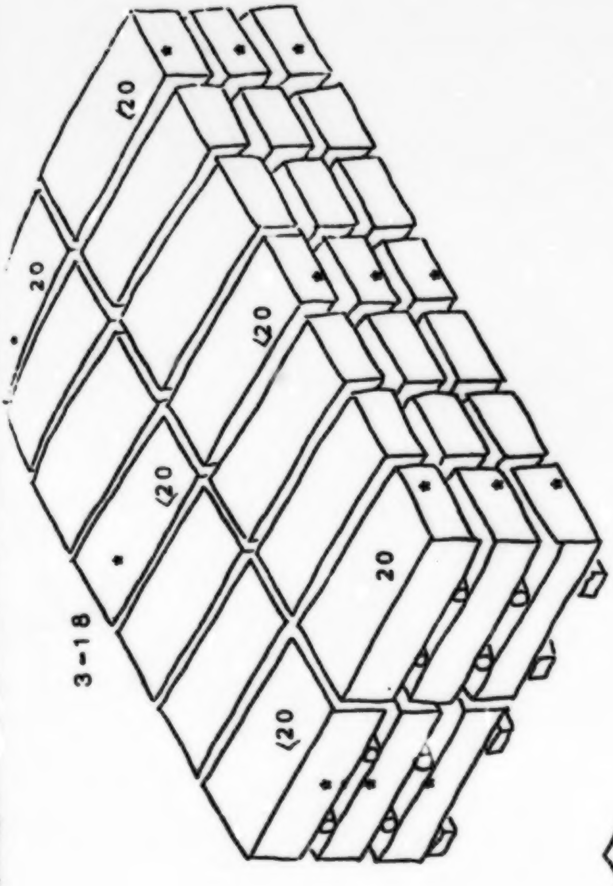
170

3-18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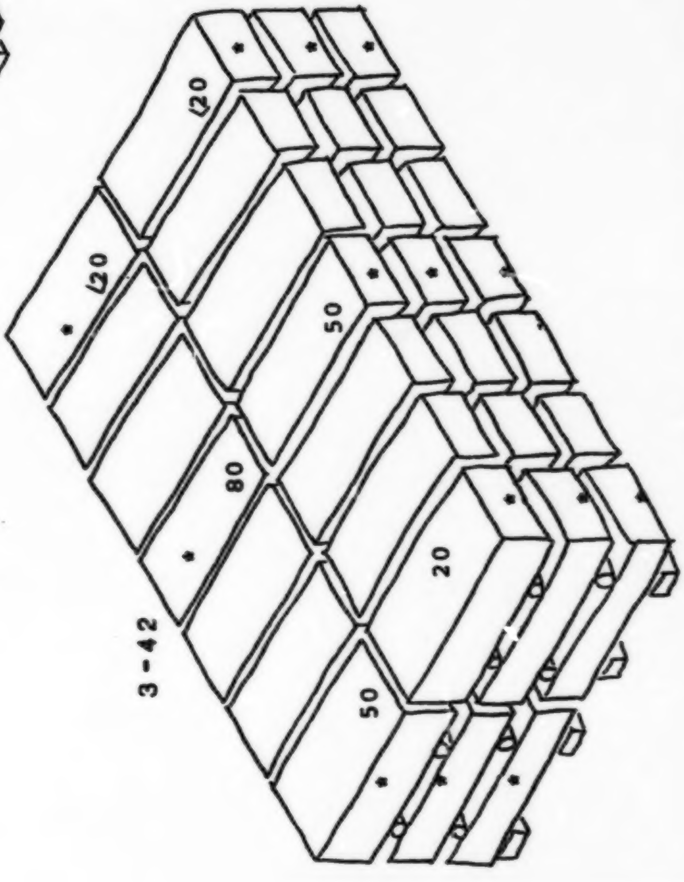
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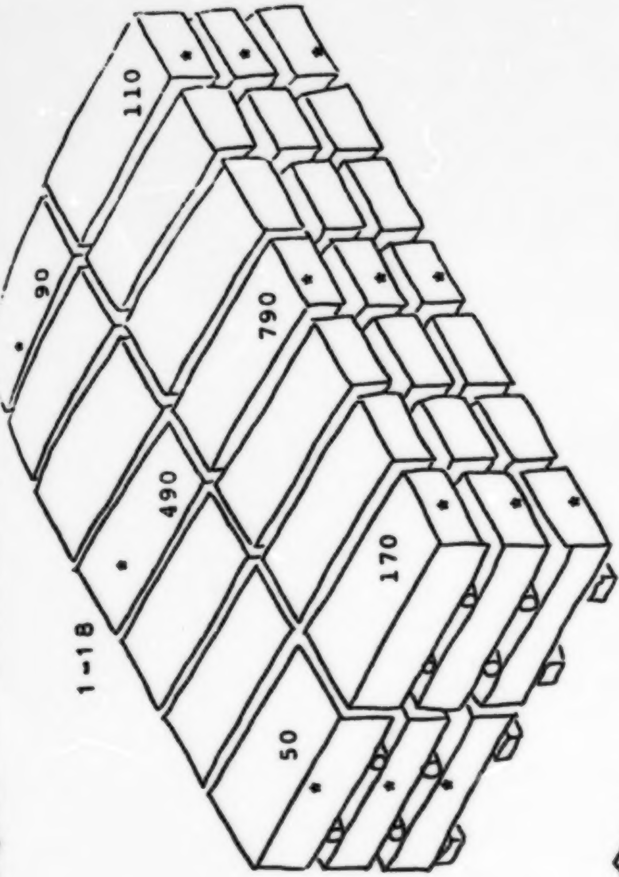

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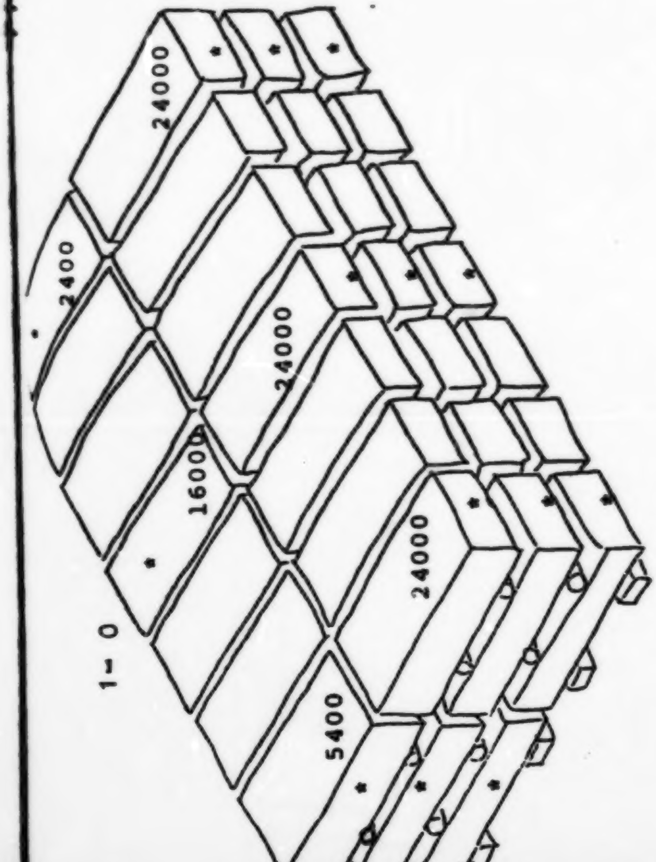
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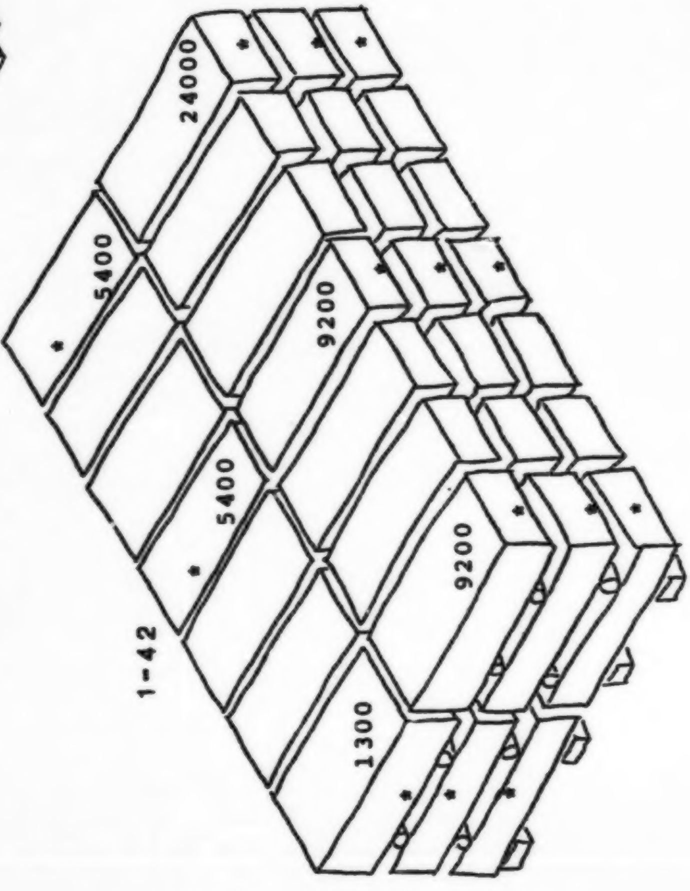
1- 0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1-42

TRIAL 4

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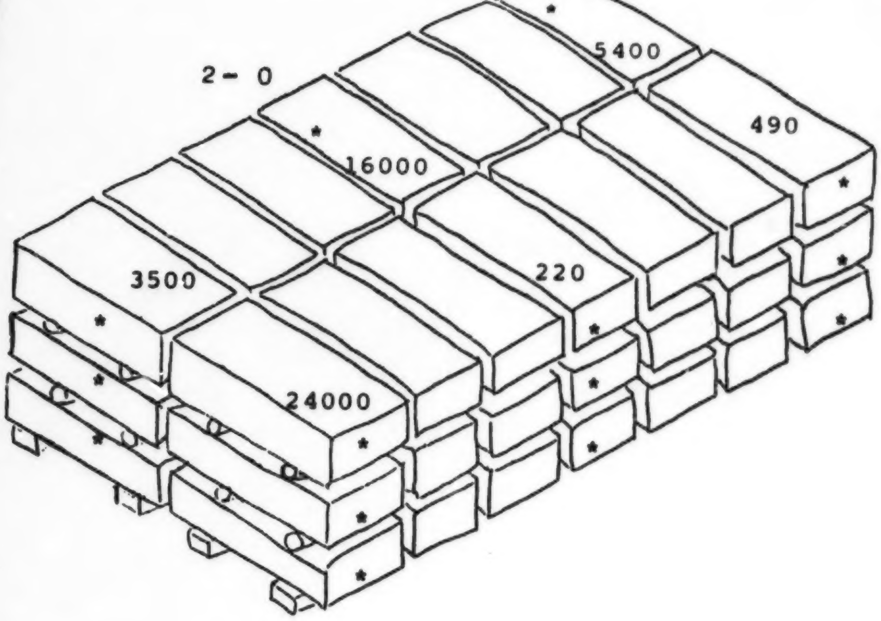
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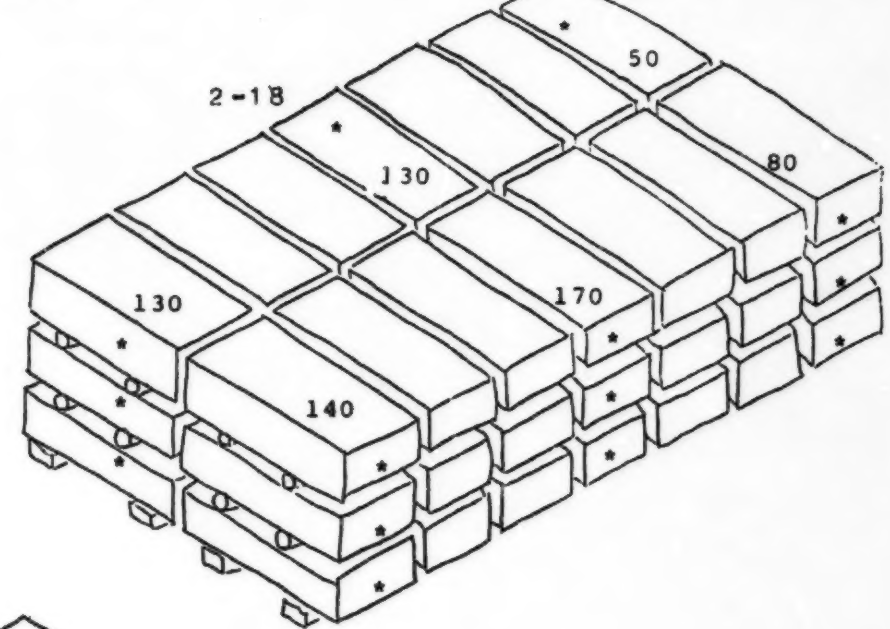
3500
220
24000

2-18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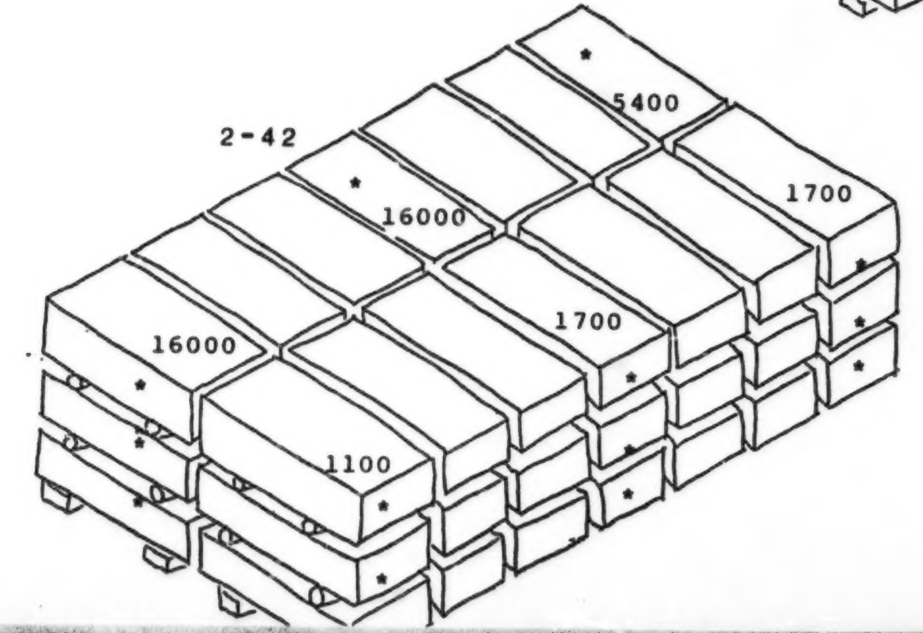
80
130
130
170
140

2-42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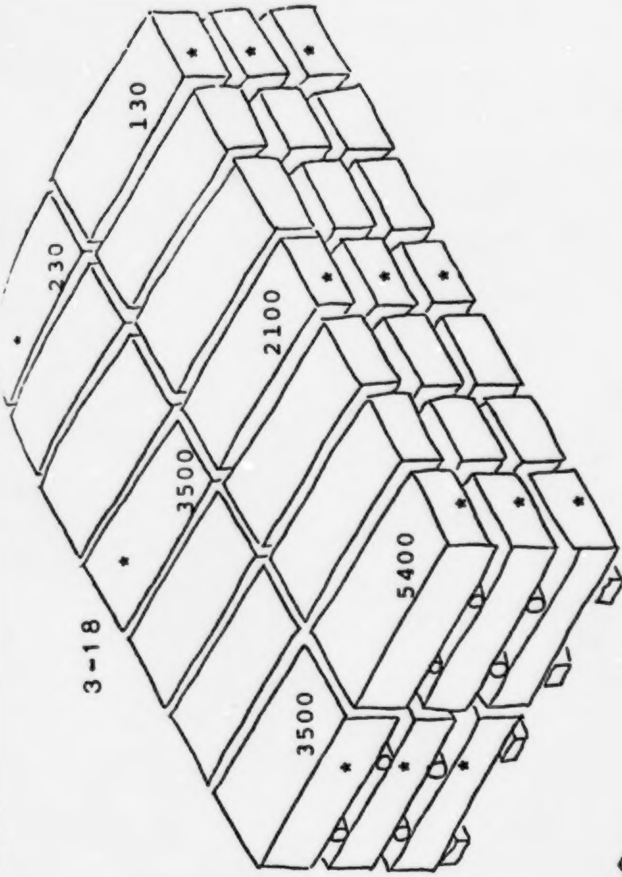
1700
16000
1700
16000
1100

TRIAL 4

3-18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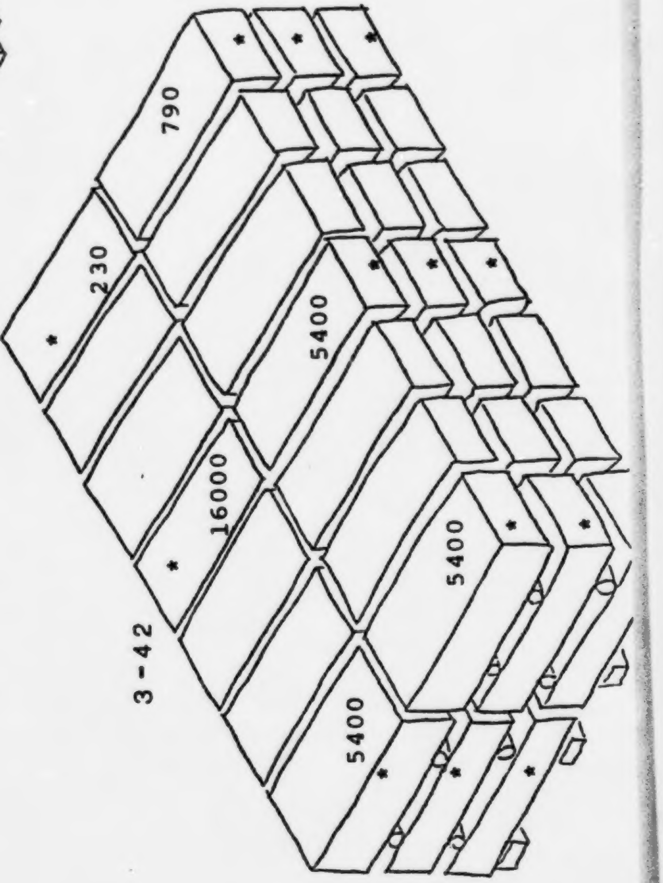
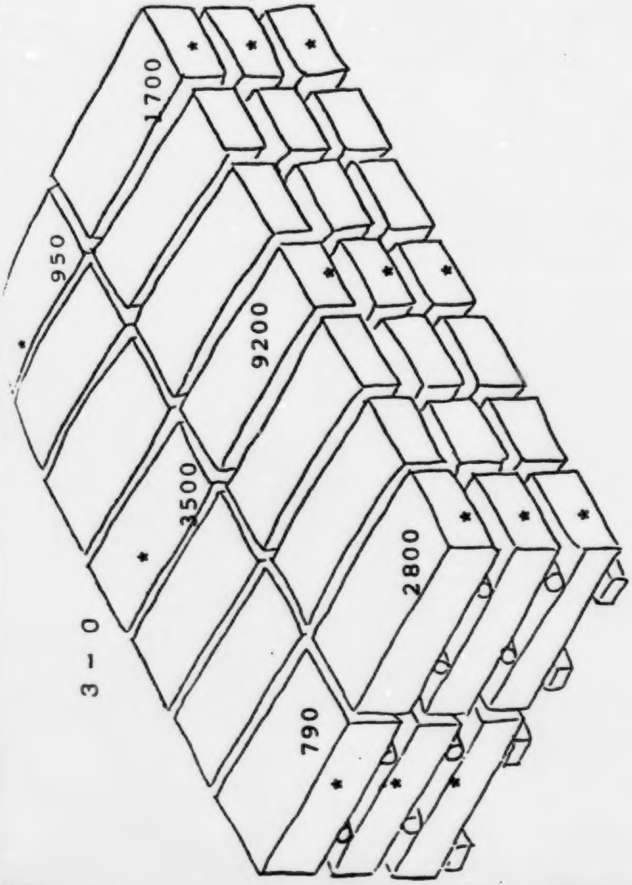
230
2100
3500
5400
3500

3-42


790
230
5400
16000
5400
5400

3 - 0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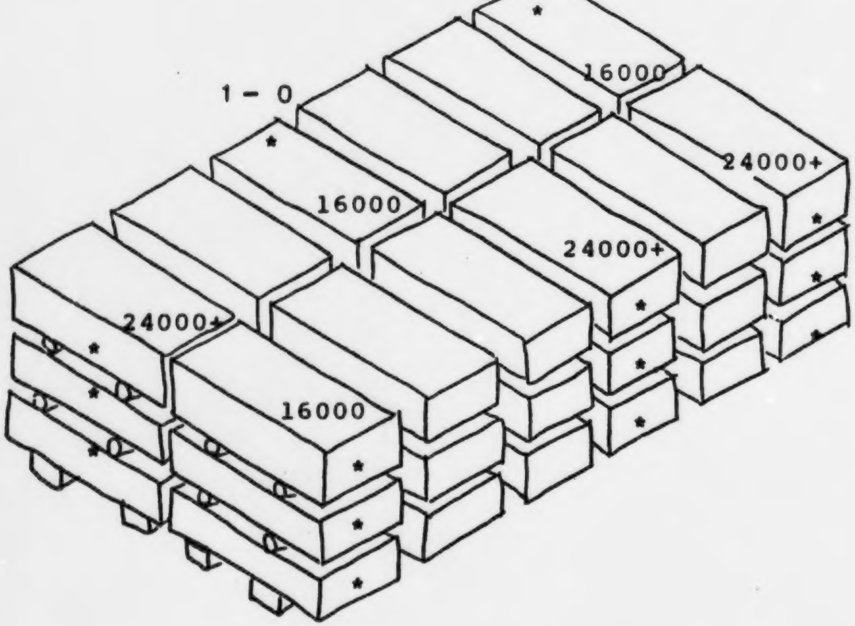
950
9200
3500
2800
790

TRIAL 5

1- 0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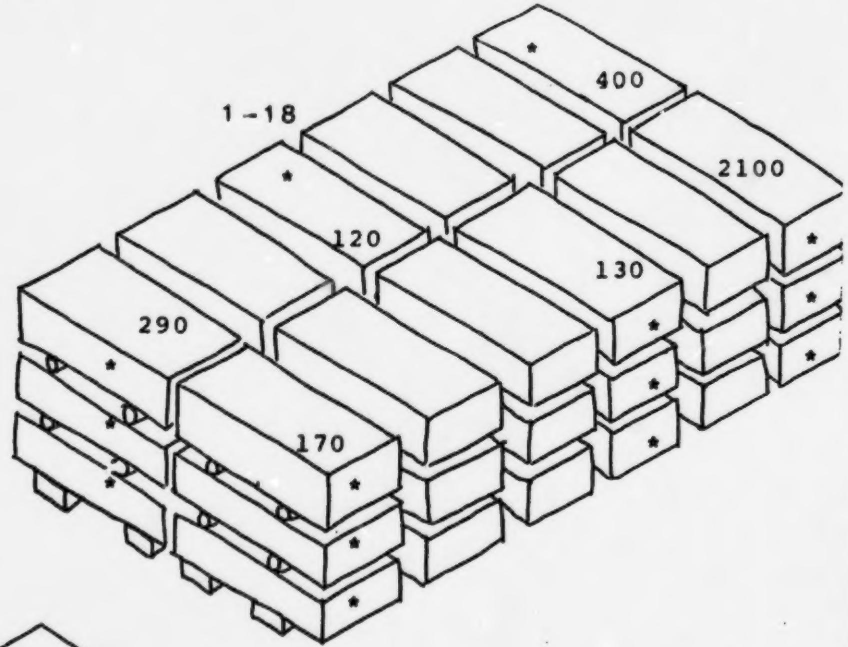
16000
24000+
24000+
24000+
16000

1-18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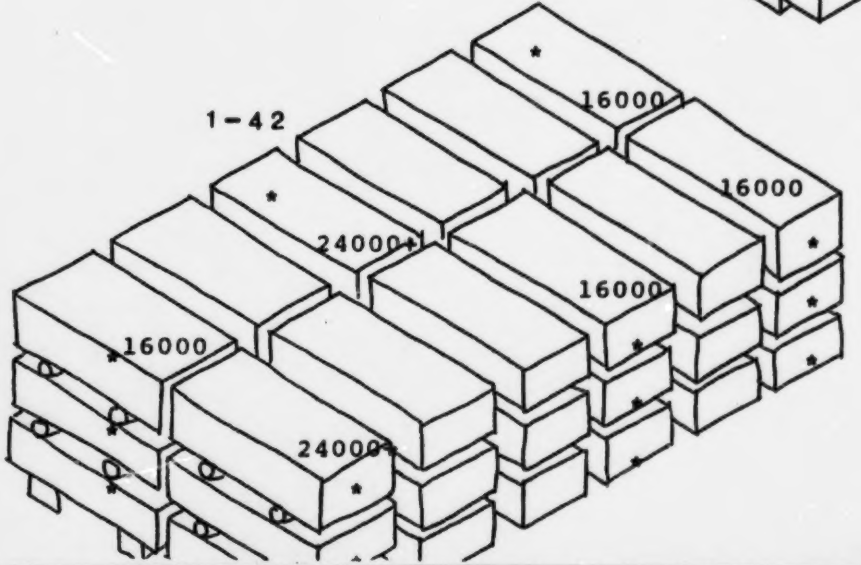
2100
120
130
290
170

1-42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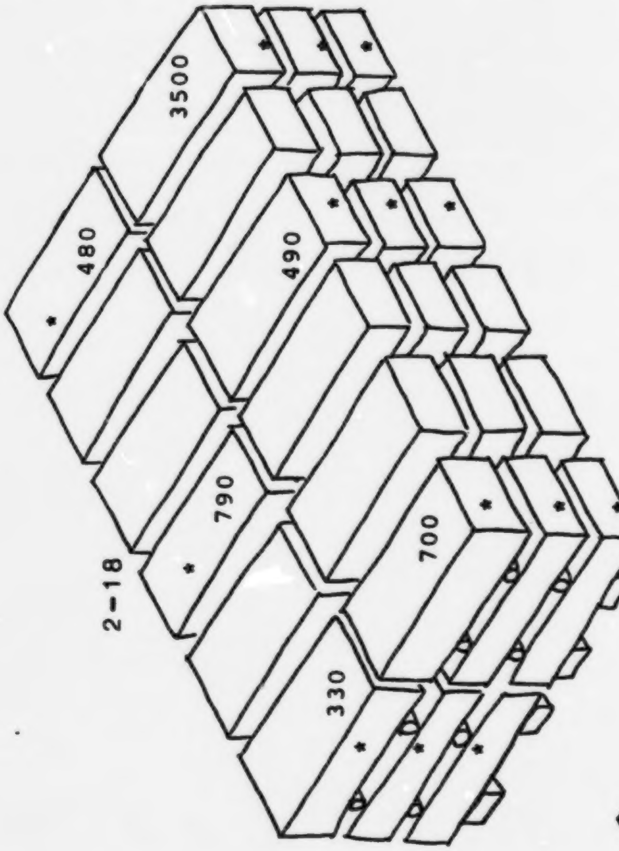
24000+
16000
16000
24000+

TRIAL 5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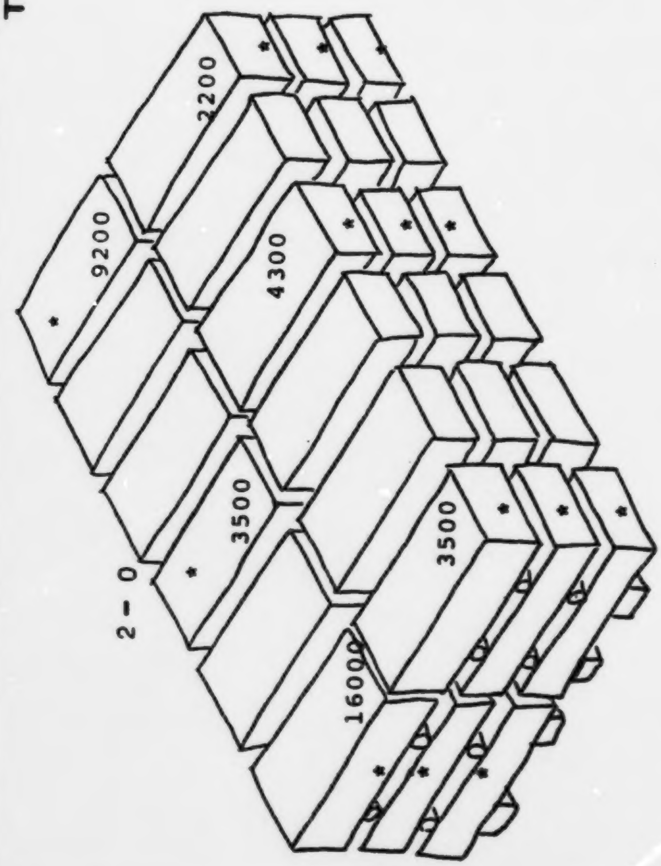
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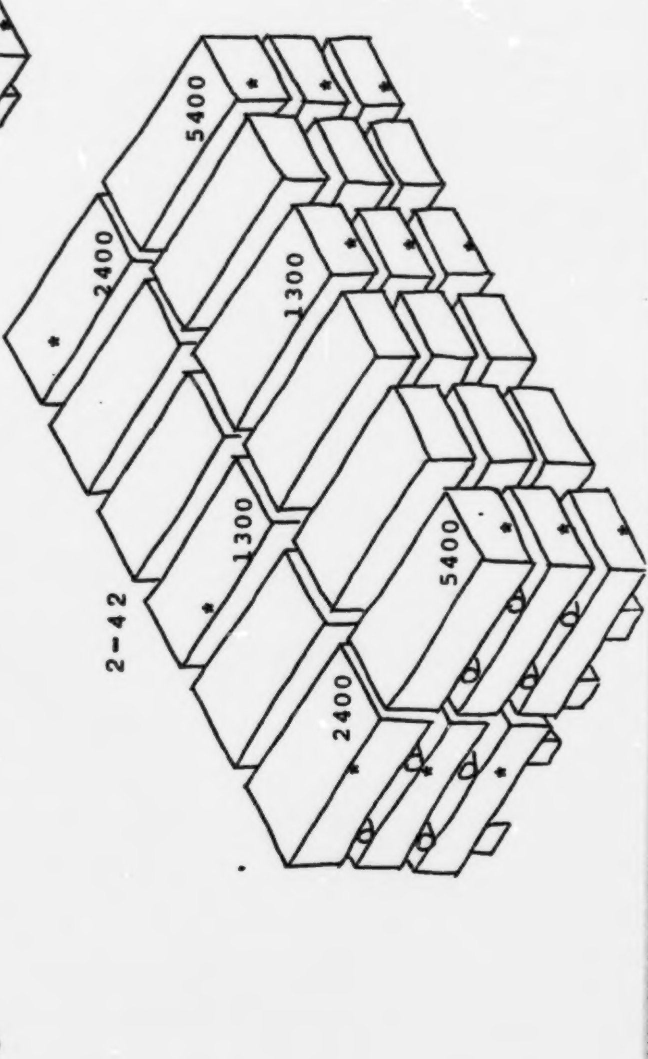
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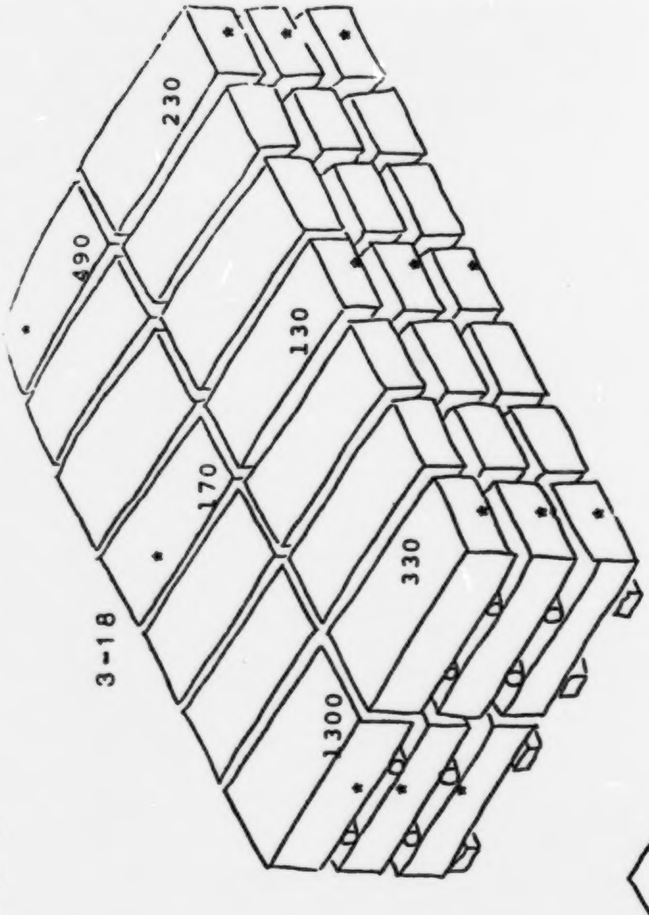
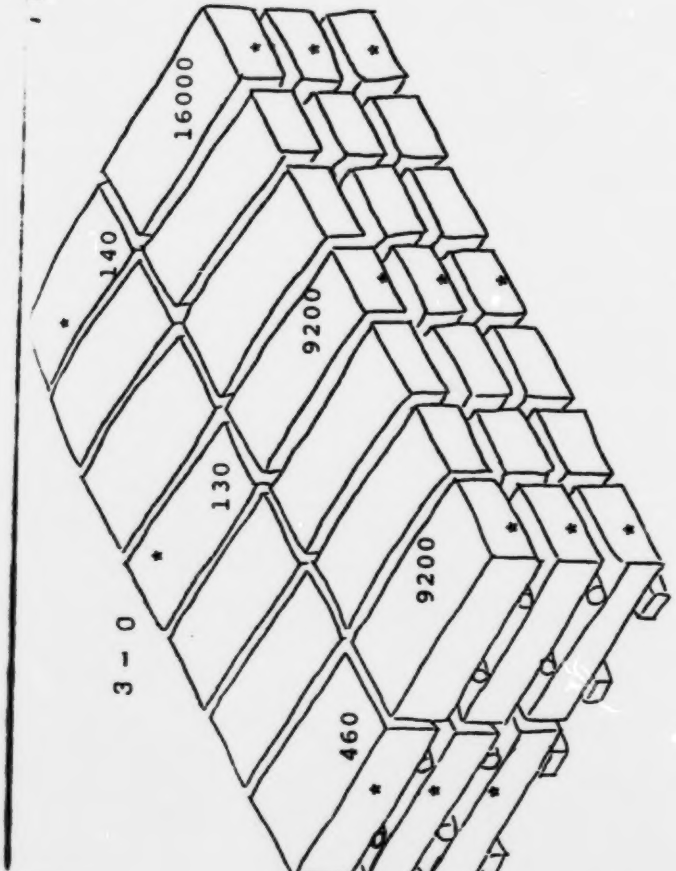
TRIAL 5

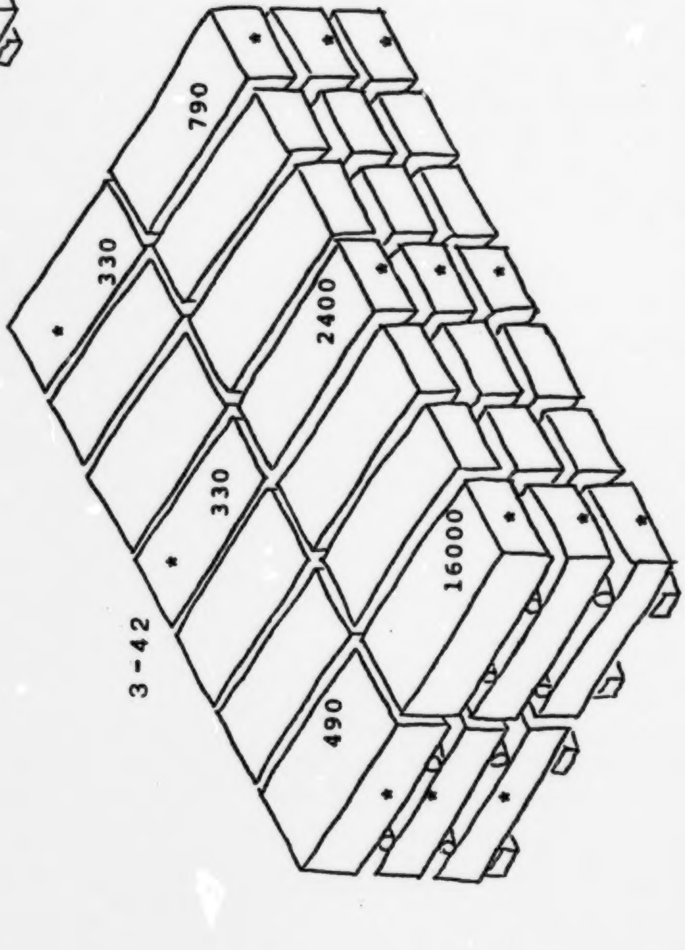
3-18


490
230
130
170
330
1300

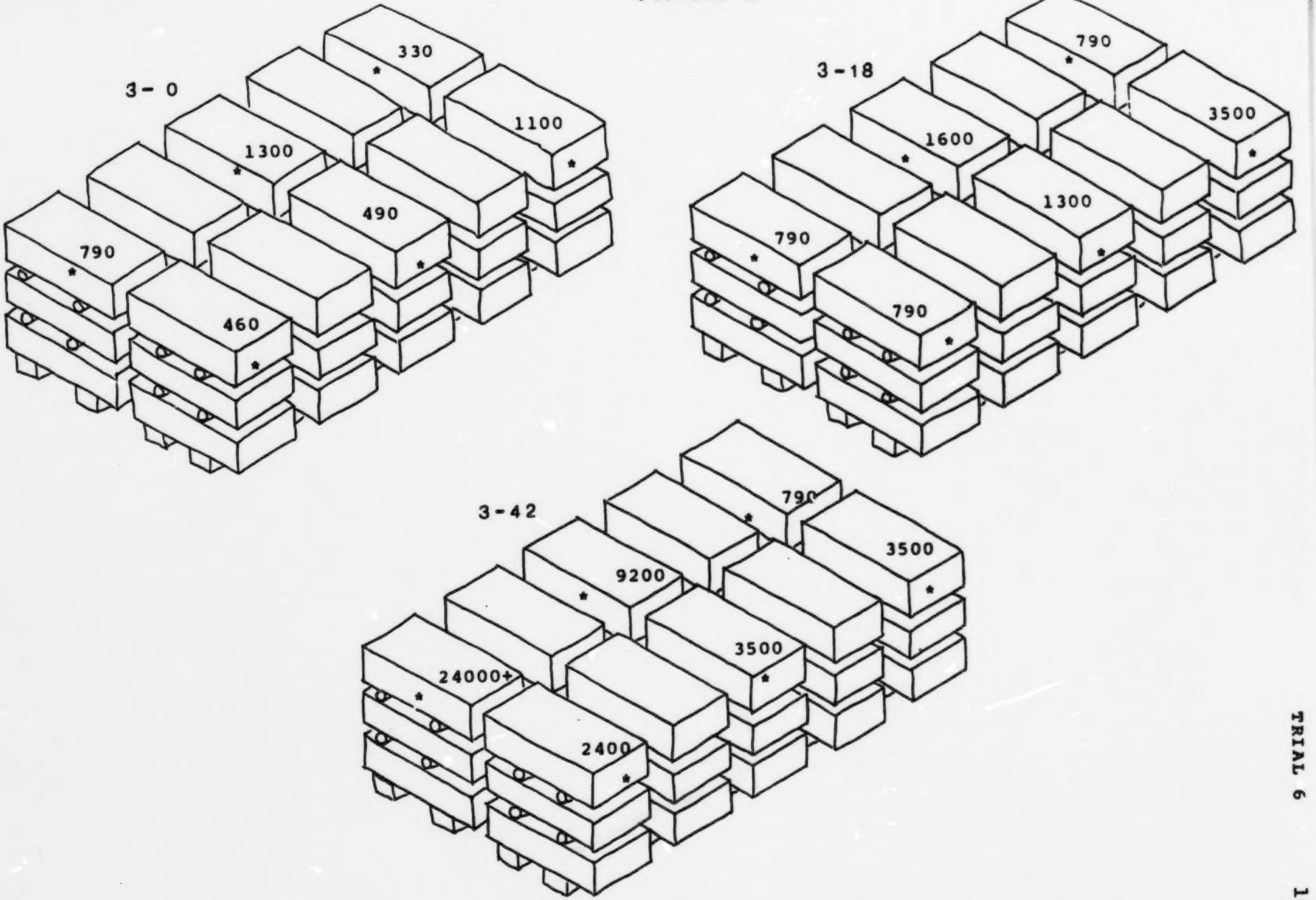
3 - 0

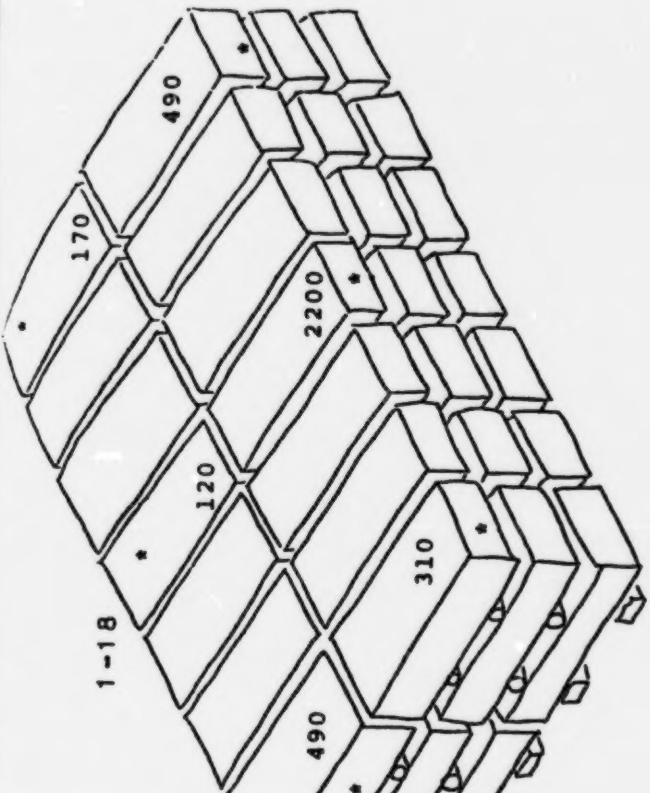

140
16000
9200
130
9200
460

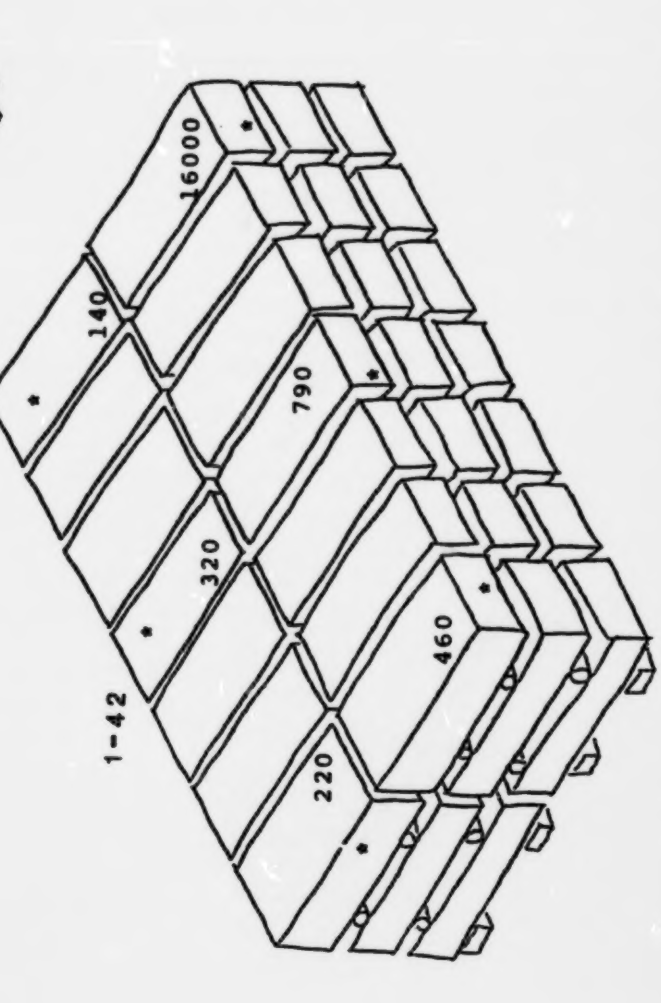
3 -42

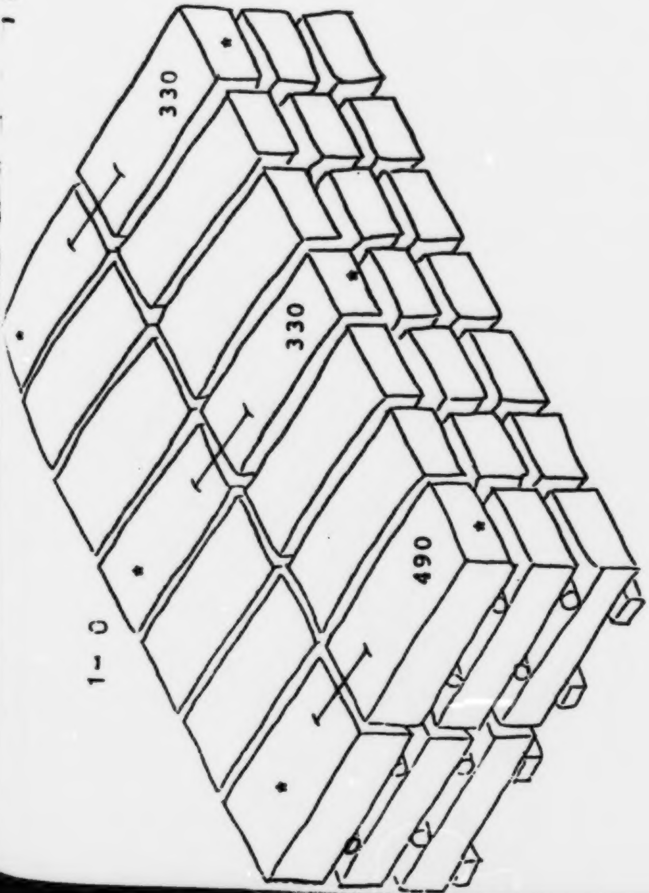

330
790
2400
330
16000
490

TRIAL 6


3- 0
3-18
1600
3-42
24000+


1-18
490
170
2200
120
310
490


1-42
16000
140
790
320
460
220


1- 0

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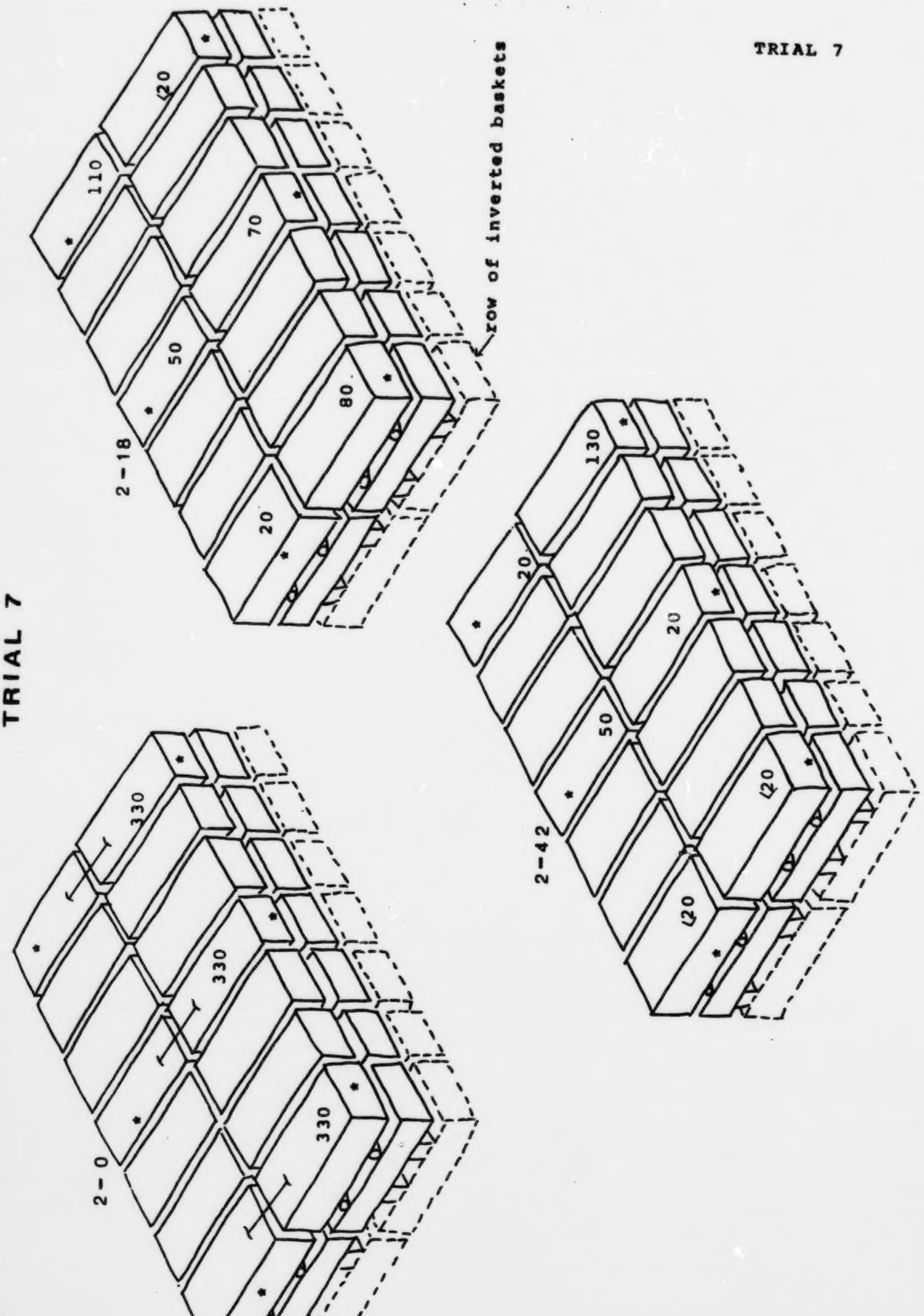
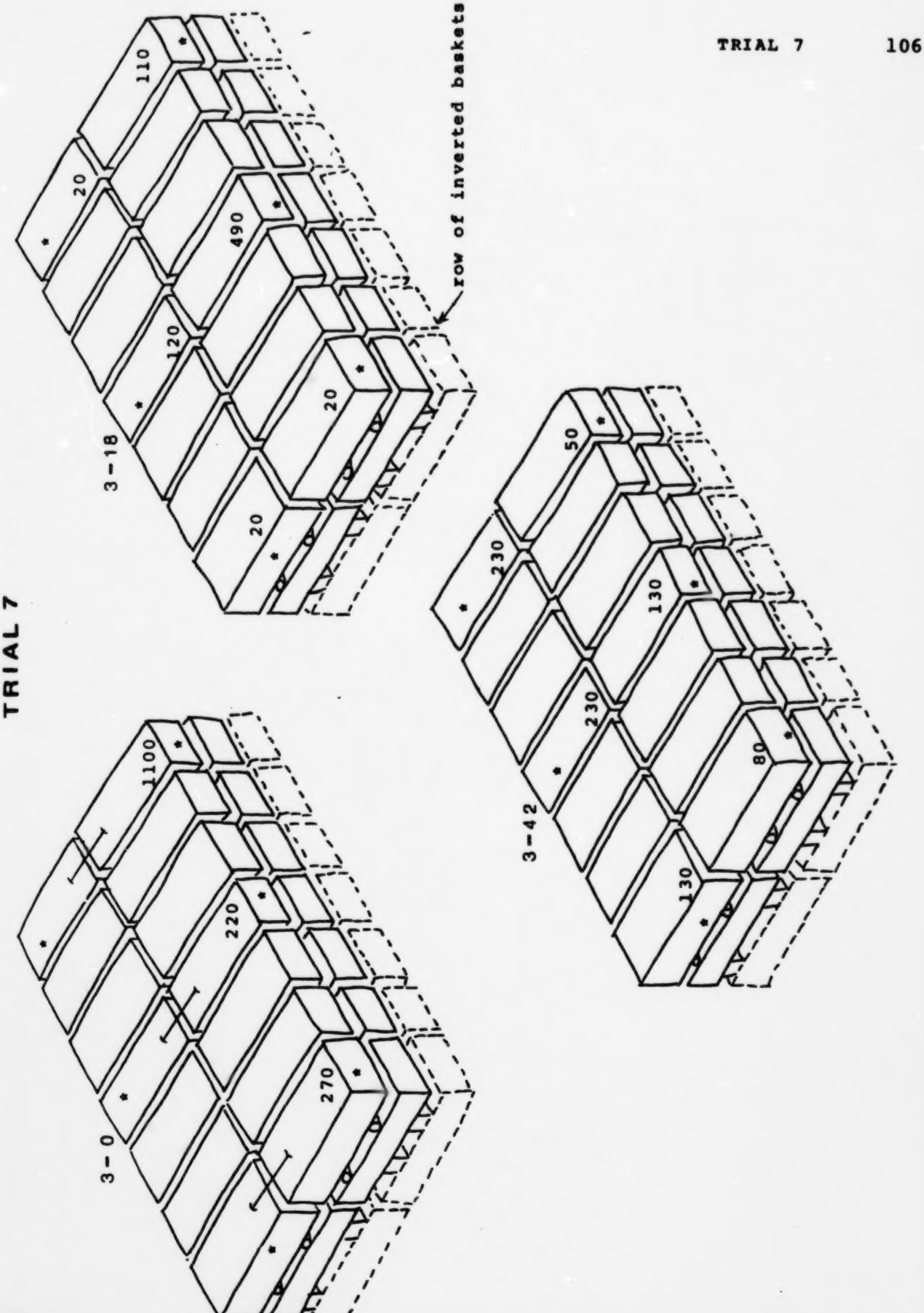
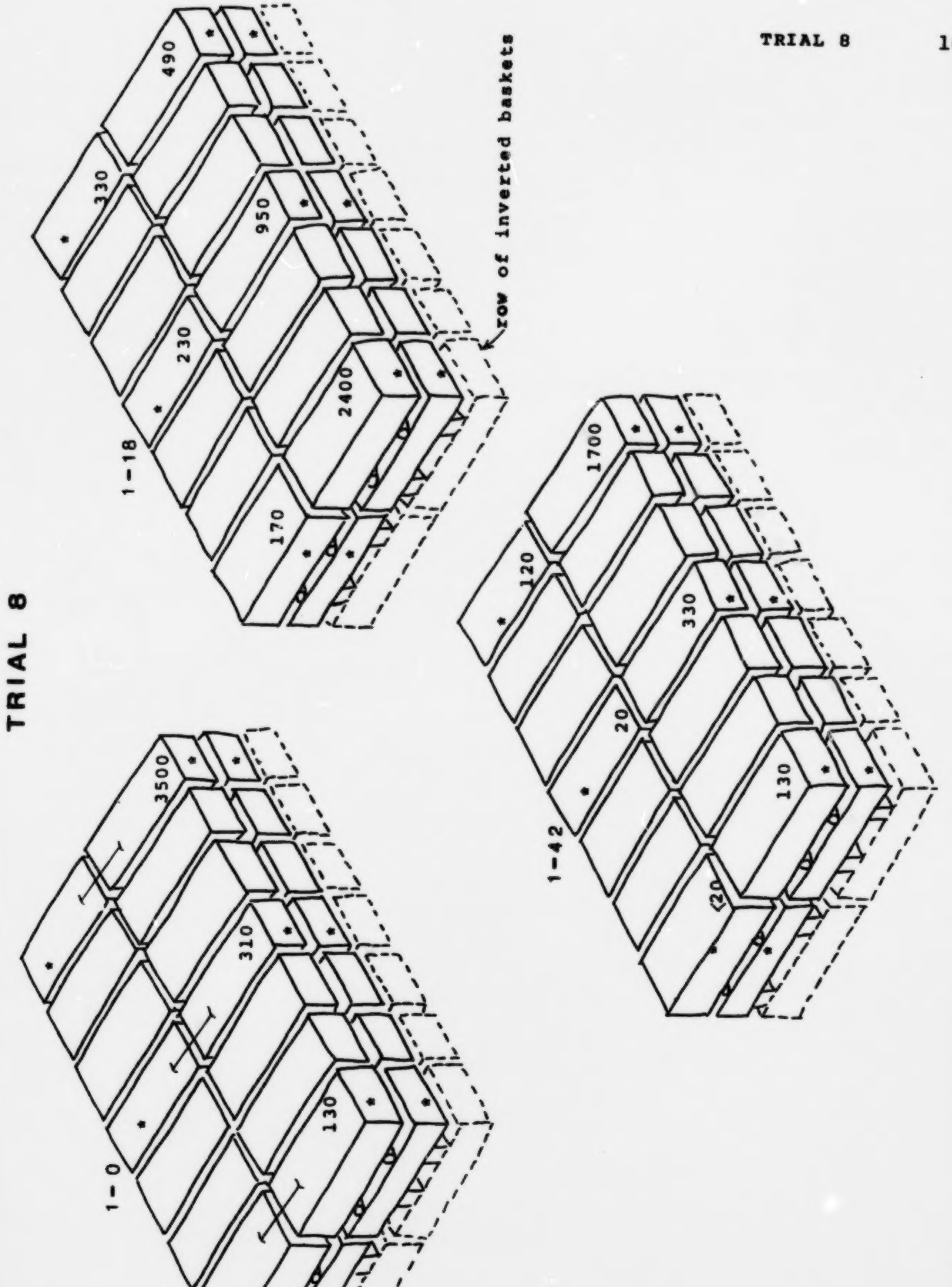

2-18
row of inverted baskets
2-0
2-42



TRIAL 7

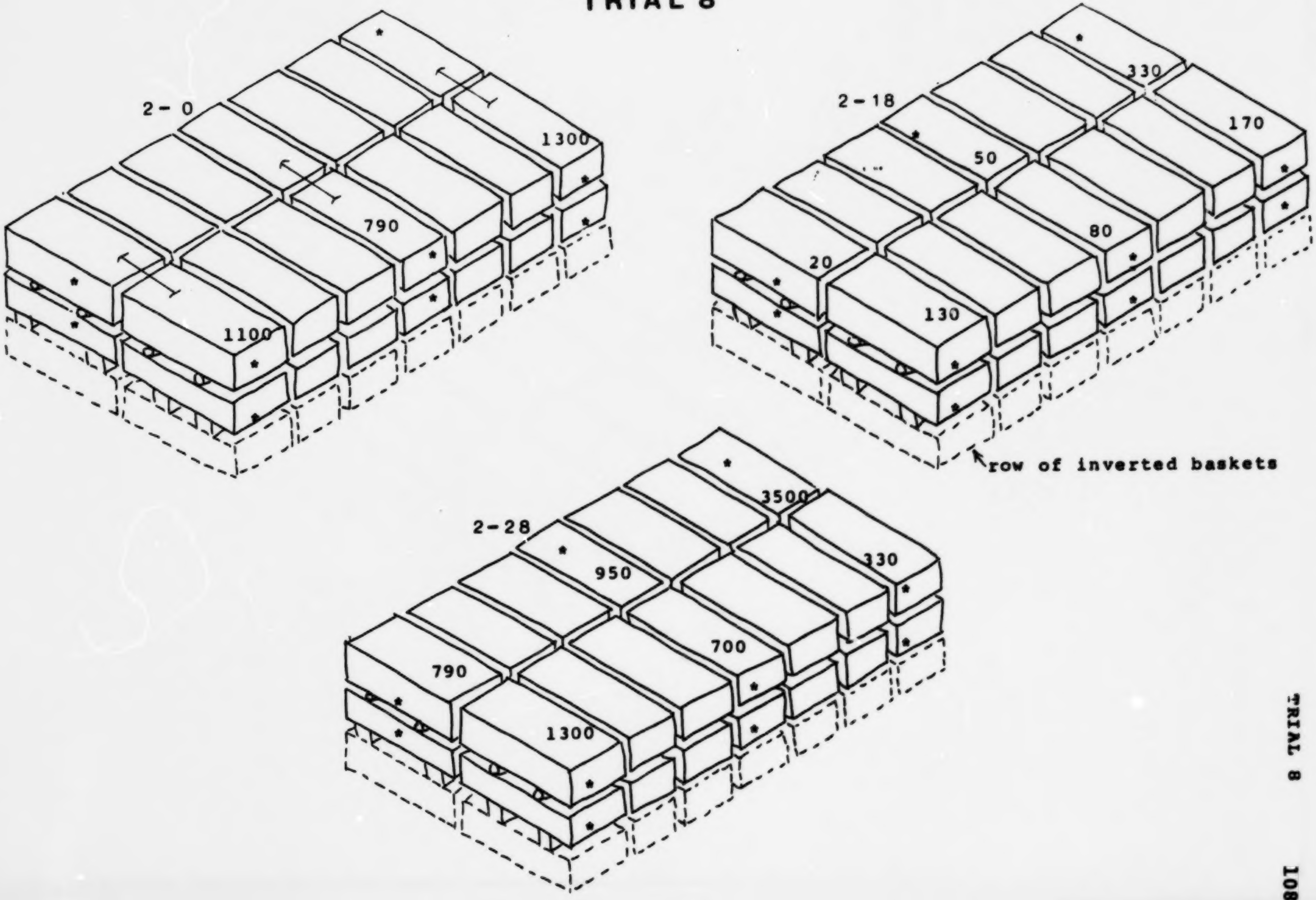
3-18
row of inverted baskets
3-0
3-42



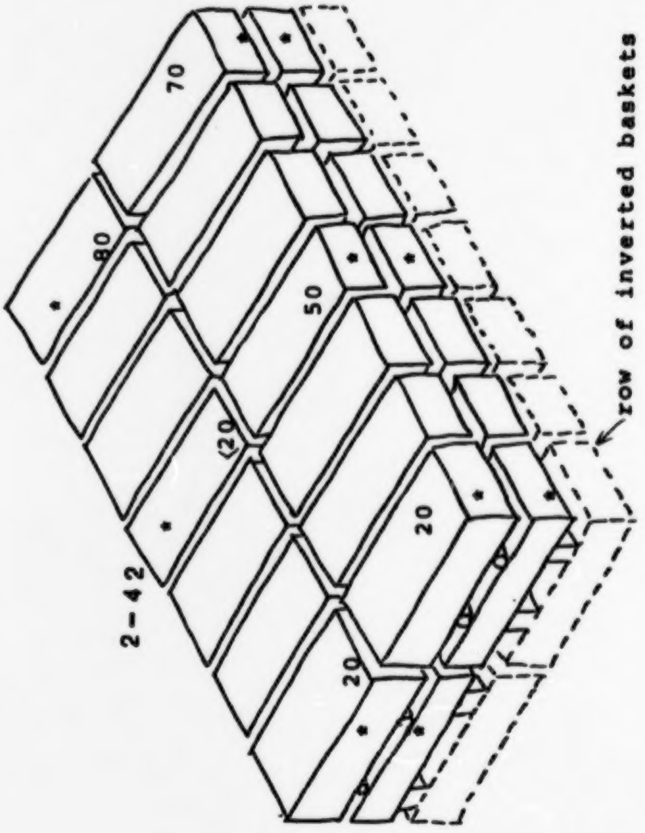
TRIAL 8

1-18
row of inverted baskets
1-0
1-42
1700

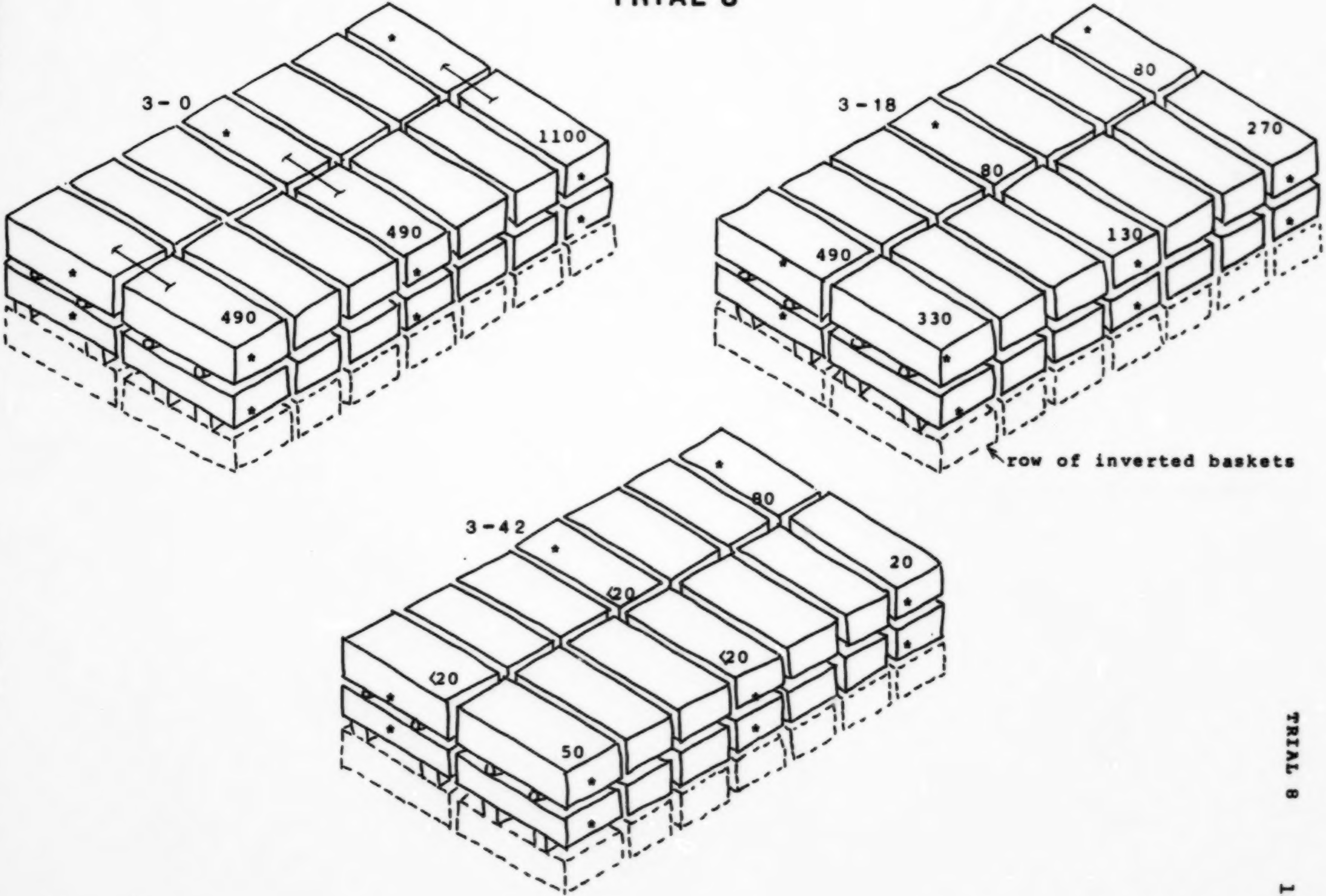
TRIAL 8

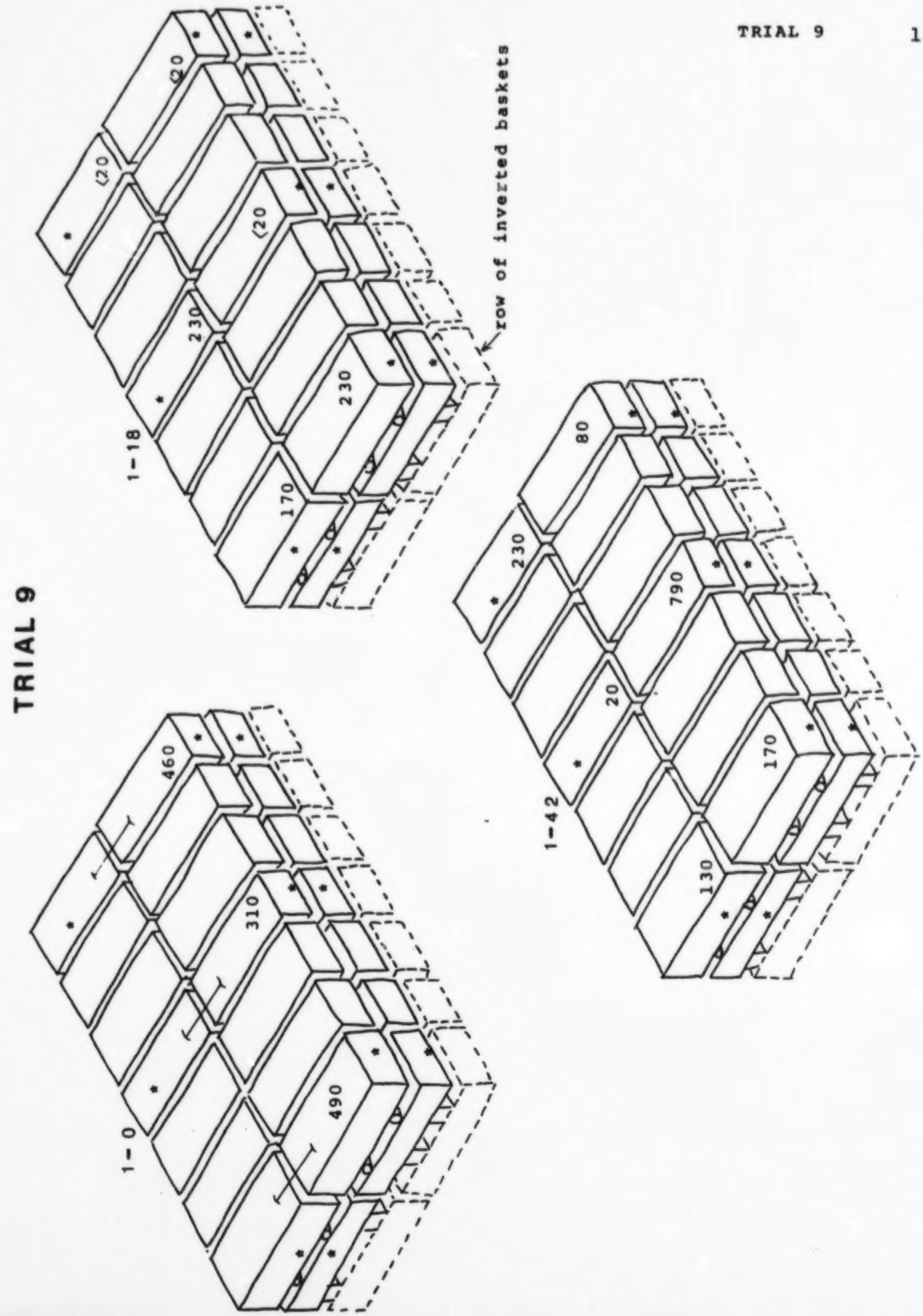

2- 0
2-18
row of inverted baskets
2-28

TRIAL 8


2-42
70
80
50
20
20
20
20
row of inverted baskets

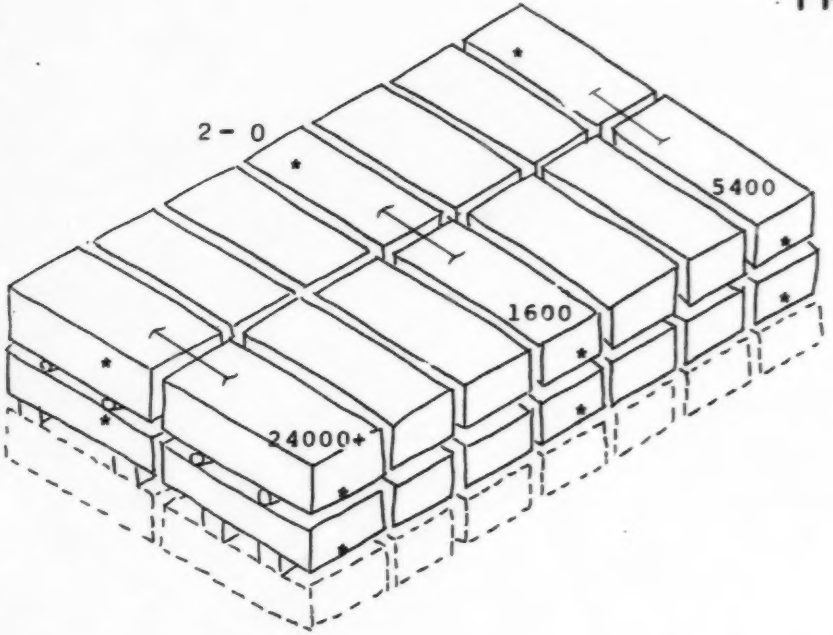
TRIAL 8


3- 0
3-18
row of inverted baskets
3-42
<20
<20
<20

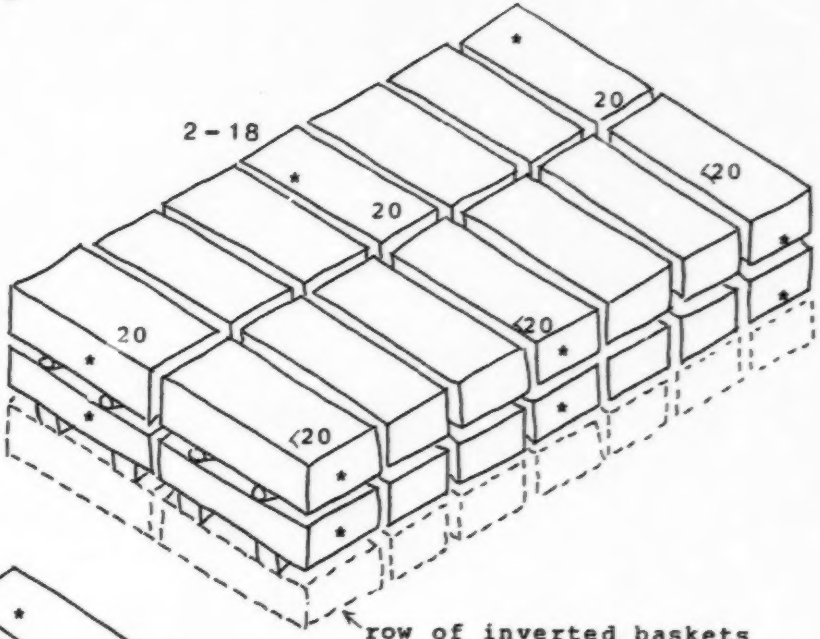

TRIAL 9
1-18
(20
(20
(20
row of inverted baskets
1-0
1-42

TRIAL 9

2- 0

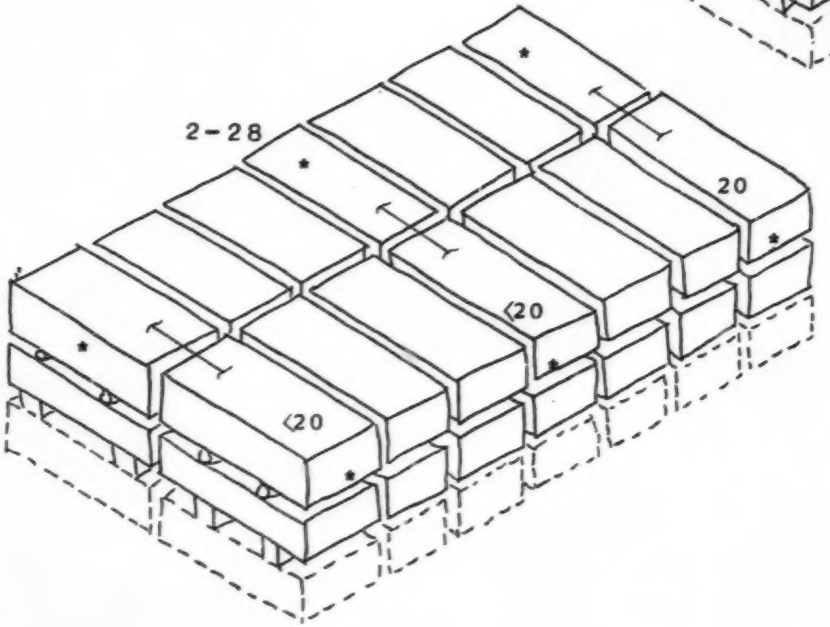

5400
1600
24000+

2-18


20
<20
20
20
<20
<20

row of inverted baskets

2-28



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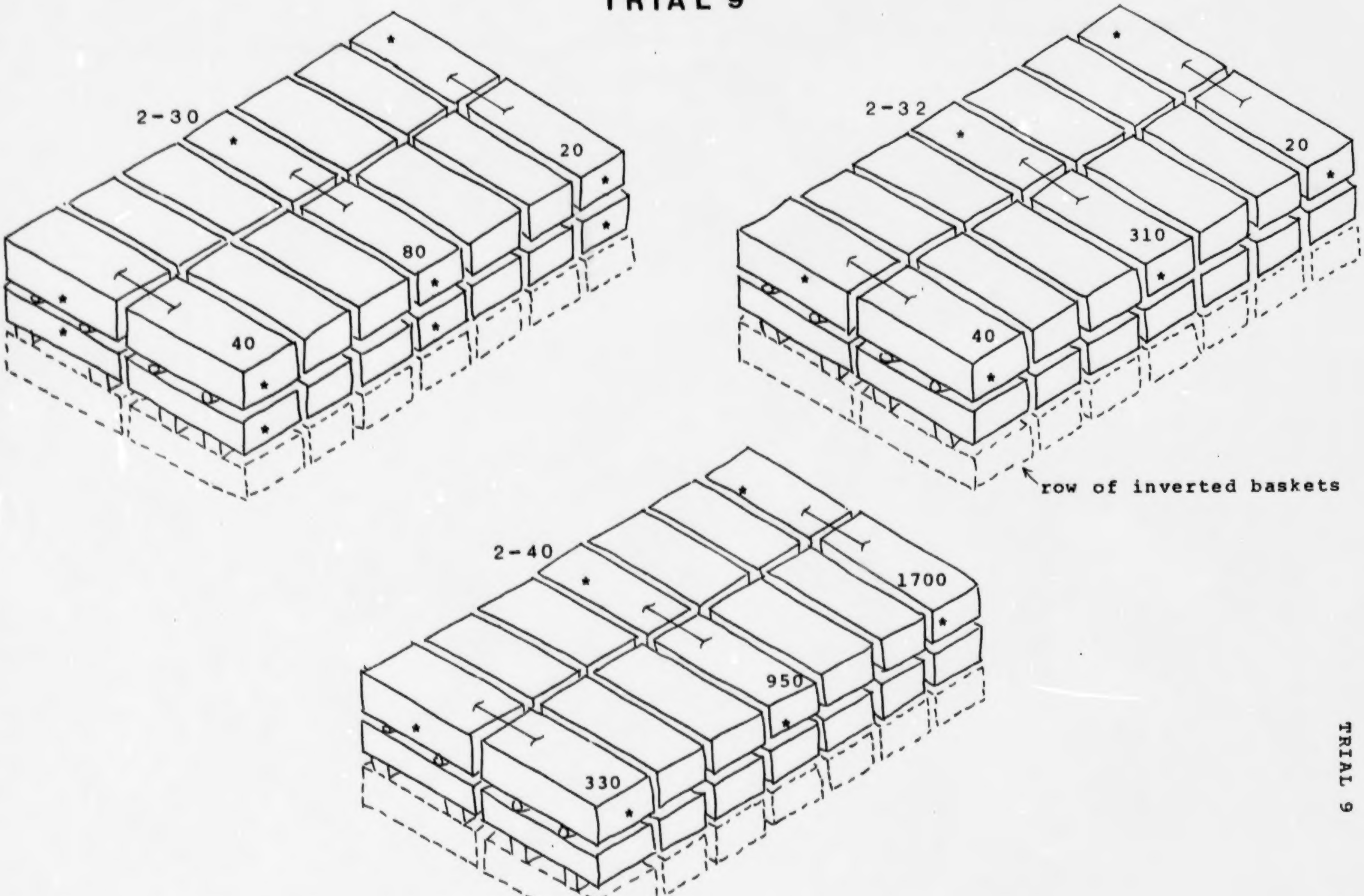
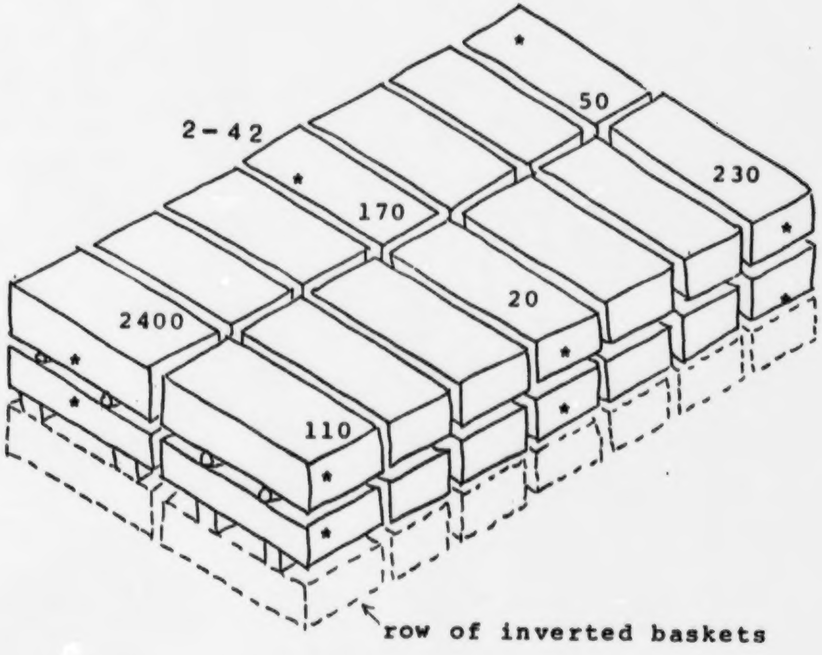
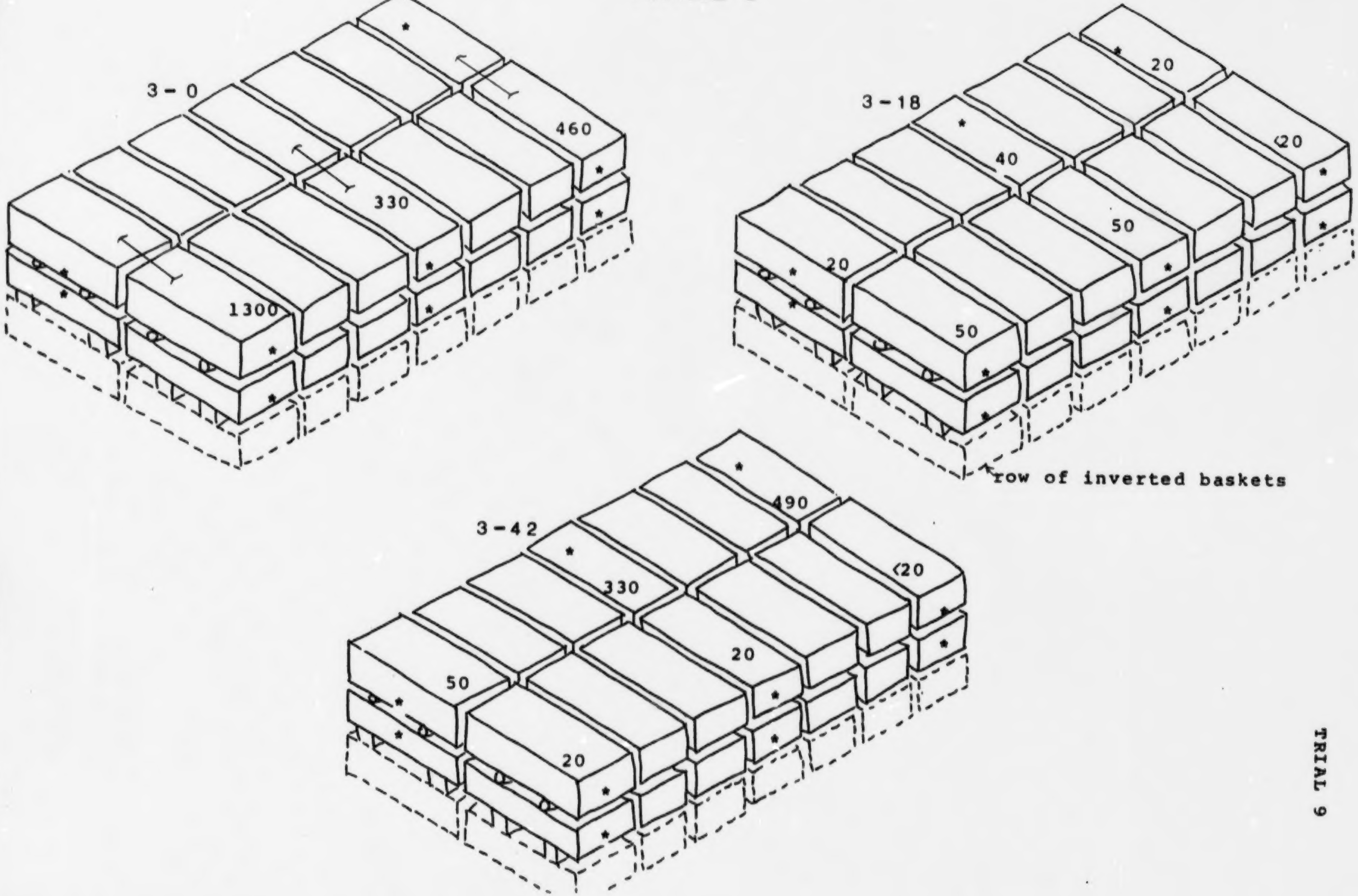

2-30
2-32
row of inverted baskets
2-40
1700

TRIAL 9


2-42
*
50
230
*
*
*
170
20
*
*
2400
*
*
110
*
*
row of inverted baskets

TRIAL 9


3 - 0
460
330
1300
3 - 18
20
<20
40
50
20
50
row of inverted baskets
3 - 42
490
<20
330
20
50
20

TRIAL 9

APPENDIX E

TANK PERFORMANCE

Ideally, the water flow in a depuration tank should be distributed uniformly. Whatever tank in a multiple tank system or whatever location in a particular tank the clams are occupying, physica conditions should be uniform and encourage pumping by the clams. Putting aside individual clam variability, clams submitted to the same conditions, as each lot is, should end up with the same bacterial loading wherever they are located in the tank.

The study of the distribution of the water flow in the depuration tank may be approached by two techniques. Dye may be added to the water entering the tank and its dispersion, with time, observed Faecal coliform count reduction, in clams from various locations in the tank, may also be used as an indicator of the water flow pattern. If a depuration tank has good dispersion and evenness of flow throughout, the clam counts from any location within that tank should differ very little after a depuration cycle. Once the hydraulics of the tank have been proven reliable, differences in faecal coliform reduction can only be attributed to the quality of the test procedures and to individual clam variability. When differences in clam counts are severe, the "bouncing phenomena" mentioned earlier is likely taking place.

Dye Studies

Several dye tests were conducted during the course of the trials. The dye used was fluorescein, a fluorescent water-soluble dye (Sodium Merk). It was inoculated in the water at the flow-meter box about one hour before the end of the depuration cycle. Color pictures were taken to record the progression of the dyed water. The rate of dye mixing varied in accordance with the flow-rate set for the particular tank system and spread through the tank in a sequential manner according to the water flow pattern.

Trial 2 was run in order to determine the uniformity of depuration throughout the tank. After examination of the bacteriological results, it was suspected that the flow was not properly balanced in the tank; more going to the right side than to the left. During this trial, the water entering the tank was distributed fro an elbow on the pipe behind the baffle (Fig. 8). In order to visually check the distribution of flow with this type of inlet, a dye study was performed near the end of trial 3. During the experimen all tanks were loaded with the same number of baskets per layer (14 Tanks 1 and 2 had a flow-rate of 1 gpm/bu while tank 3 was set at 0.5 gpm/bu. Tanks 2 and 3 were left with the water distribution system unchanged (elbow on pipe) while a 6-inch extension pipe was added to the elbow in tank 1. In all tanks, the dye progressed along the bottom and up at the outlet end, then up at the sides and centre, and finally up at the inlet end. During this trial, the baskets were packed close against the baffle at the inlet end leaving a 5-inch space near the outlet end. This may explain in part why the dye showed first at the outlet end. In tank 1, the water was coming up evenly across the width of the tank at the outlet end but in the mid-section of the tank it was coming up to the left side faster than to the right. In tank 2, there was an uneven distribution of the dye both across the outlet end and across the mid-section of the tank. Near the outlet the dyed water first appeared on the right, then centre and left; along the sides of the tank it first appeared on the right, then centre and left. The same unbalanced pattern repeated itself in tank 3. The extension pipe on the elbow (tank 1) seemed to help balance the flow, at least close to the outlets.

Following this experiment, an extension pipe was also added to tanks 2 and 3 to improve the flow distribution.

Another dye study was performed near the end of trial 6. Only tank 3 was used during this trial. The flow-rate was established at 0.5 gpm/bu. Baskets were disposed in 3 layers of 10 baskets each. The use of 10-basket layers instead of 14 allowed greater spacing between the side-walls of the baskets and changed the flow pattern in the tank. Now the dyed water travelled along the bottom, coming up in the centre of the tank first, then at the outlet end, and last, at the inlet end. No observations were made as to flow balance between the two sides of the tank. This spacing may have improved the uniformity of the flow distribution (water spreading out in all directions from the centre of the tank?) and the quality of depuration, however, bacteriological results of that trial cannot corroborate this hypothesis. Bacterial counts increased steadily throughout the trial; the effect of this particular arrangement of baskets cannot be separated from the effects of not having a mid-cycle drainage or a hosing down of tank and baskets (operating procedures used during the trial).

These limited dye experiments indicate variability in the water circulation pattern within the tanks. The addition of an extension pipe to the elbow pipe behind the baffle and the provision of greater spacing between baskets seemed to give a slight improvement in the flow distribution. However, the water at the inlet end of the tank was still renewed more slowly than in the rest of the tank. The flow was not evenly distributed between the left and right sides of the tank.

The tanks bulged on all sides and on the bottom when loaded to full capacity (21 bu.) and filled with water. This distortion may lead to a preferential flow pattern following the curved bottom and sides of the tank. An unleveled tank position would also cause an uneven flow distribution between the two sides of the tank. Another possible cause of uneven distribution might be the angle and position of the elbow and extension pipe with respect to the tank. A slight inclination of the pipe to one side of the perpendicular would not allow equal spreading of the water. An ideal hydraulics system in the tank would require considerable experimentation with tank shape and water delivery and outlet design.

Faecal Coliform Counts as an Indicator of Flow Pattern

Tank performance may be examined quite extensively using bacteriological counts. A wide variety of comparative relations of these counts may be established between different tanks, between times of depuration, between different tanks at different times, etc. Particular locations within one tank or among many tanks may also be investigated.

The bacteriological counts from the three depuration tanks used provide interesting data on their performance and variability. Table E-1 illustrates a few of the possible approaches to examining tank variability.

When all bacteriological data are pooled as an overall array, there are no significant differences in the median values from the locations sampled at 18-24 Hour. However, at the second sampling time (42-48 Hour) the median value from the middle locations (295 MPN) is greater than the "in" and "out" values. This indicates that, in general, clams from the middle location of the depuration tanks would not reduce their bacteria load as much as clams located at the ends of the tanks.

Bacteriological results may be grouped to allow study of tank-to-tank variability and, according to the locations sampled in the tank, variability may be studied cross-wise, i.e., perpendicular to the water flow, sideways, i.e., parallel to the water flow, or both simultaneously. Faecal coliform counts indicate that as a general rule tank 2 was more reliable than tanks 1 or 3. There was less variation between the counts from different locations within tank 2 than between counts within the other two tanks. Not only were the counts more uniform in tank 2, they were also lower compared to the counts in other tanks. Therefore, clams placed in tank 2 had a better chance to depurate to meet the criteria set for depurated shellfish than those put in tanks 1 or 3. The cross-wise analysis of counts shows that clams located at the "in" end of tank 3 were depurating rather poorly for the first 18-24 hours. By 42-48 hours of depuration the poor performance had shifted to the "mid" and "out" locations in that tank. Tank 1 gave poorer performance than tank 3. Initially, there was not a great deal of variation in tank 1, as shown by the 18-24 Hour sample. Differences were, however, noticeable by 42-48 Hour. Tank 2 performance was relatively constant, with variation in counts from different locations being negligible.

When examined sideways, counts in tank 2 were still uniform, independent of sampling time. The same could not be said of tanks 1 and 3 where important variations took place. In tank 3 less bacterial reduction occurred on the left side by 42-48 Hour, while in tank 1, the depuration rate was slower and by 42-48 Hour, the counts had gone up to 790 MPN on the right side.

A combination of the cross-wise and the sideways median values gives clues on which tank and/or which location allowed clams to most reduce their bacterial load, as well as those where clams would depurate the least. The biggest variation occurred at the end of the depuration cycle (42-48 Hour sample) when clams had been exposed to the tank environment for the longest time. Clams placed in tank 2 close to either side of the intake end had the greatest chance to reduce their bacterial load. By contrast, clams placed in tank 1 in the right middle location showed the highest coliform count by the end of a 42-48 hour depuration cycle. All other tank-locations had intermediary degrees of performance according to the faecal coliform counts of the clam samples.

To study the uniformity of depuration within a tank bacteriological analysis was performed on clam samples from every basket of tank 1 at the 24 and 48 Hour sampling during the second trial (2-1). The possibilities of arraying the data from this study on the performance of tank 1 are almost endless. It is not the purpose of this report to analyse these data in an exhaustive manner. A brief examination of the counts revealed that variations in the degree of depuration within the tank showed up more clearly after 48 hours of depuration time. At this point, there was a slight difference between the right and left side of the tank. Variation was more noticeable between the arrays of counts from the different layers of clam holding baskets in the tank (Fig. E-1). The top layer was not depurating as effectively as the bottom or middle layer.

This study of tank performance reveals that a tremendous variability, caused by the unevenness of the water flow pattern in the particular tank design used during the trials, may exist in the reduction of bacteriological load by soft-shell clams. It would be worthwhile to examine this tank design in more detail and determine possible improvements which might eliminate or reduce tank-related variability.

TABLE E-1. Tank performance and variability. Data shown is the median value MPN F.C./100g from the number of samples (N) taken from a particular location in the depuration tank. Locations are: "In" bacteriological count from baskets adjacent to the tank baffle, i.e. close to the intake pipe. "Mid" count from the middle of the tank. "Out" count from baskets located near the outlet pipes.

Over-all array: The median values from the particular depuration time include all data from all tanks. "N" equals 44 for all median values shown.

18-24 Hour			42-48 Hour		
In	Mid	Out	In	Mid	Out
130	120	110	150	295	130

Tank-to-tank variability: The medians shown for the particular depuration time come from an array of counts (N) from the location and tank specified.

Tank no.	N	18-24 Hour			42-48 Hour		
		In	Mid	Out	In	Mid	Out
1	15	170	130	110	220	330	140
2	14	80	75	65	50	70	75
3	14	330	130	130	130	330	230

		18-24 Hour		42-48 Hour	
		Left	Right	Left	Right
1	18	170	200	225	790
2	21	50	80	50	50
3	21	120	130	230	80

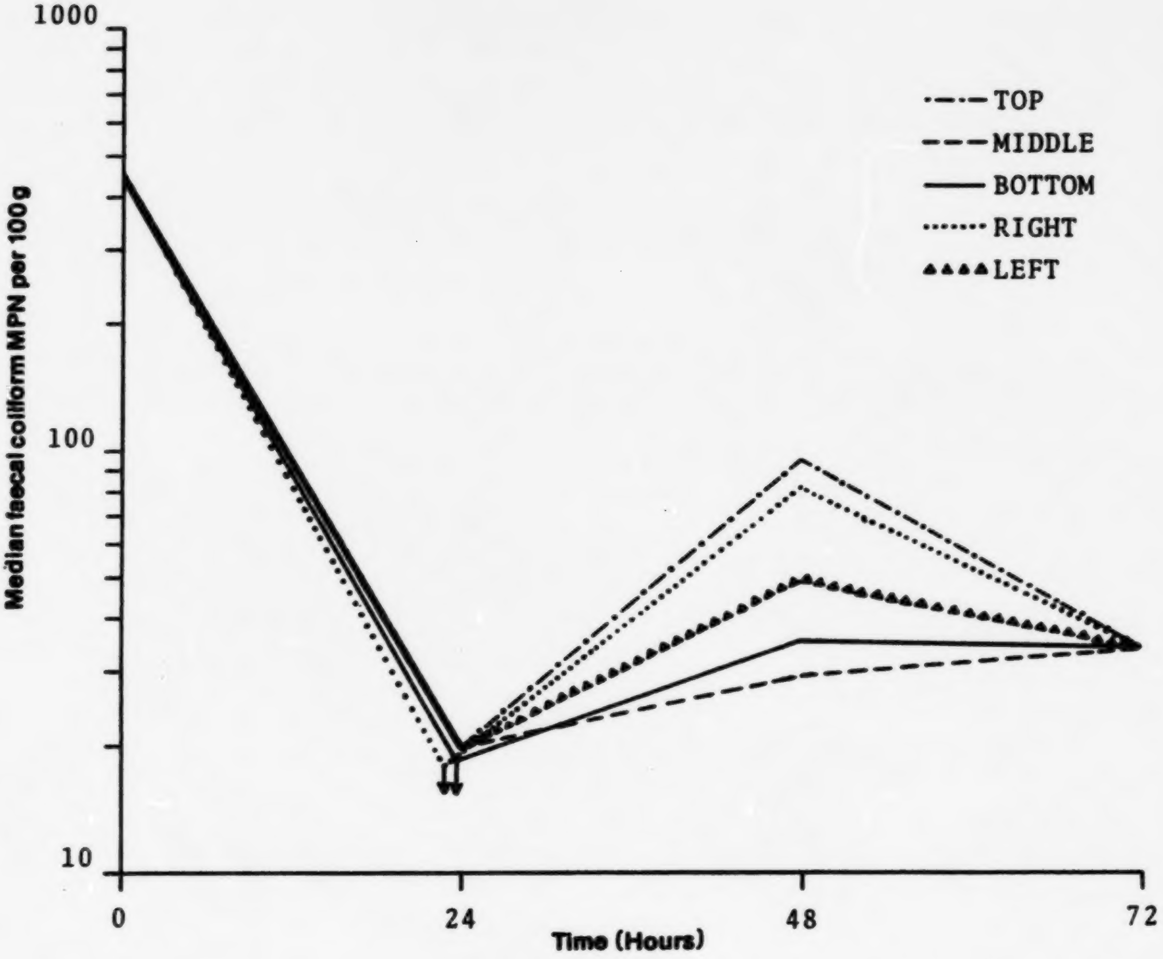


FIG. E-1. Arrays of counts showing the unevenness of depuration versus time according to the locations examined within the depuration tank.

POOR

QUALITY

ORIGINAL